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ORIGINAL CONTRIBUTIONS

Satietin, a blood-borne anorectic glycoprotein, as the putative rate-limiting satiety signal in the negative feed-back of food intake

J. Knoll

Department of Pharmacology, Semmelweis University of Medicine, Budapest, Hungary

Summary

Satietin, a 50,000 dalton anorectic glycoprotein, was isolated from human serum. Its isoelectric point is 7.0. It contains 14–15 % amino acids and 70–75 % carbohydrates. Its biological activity survives digestion with proteases and boiling.

Satietin is a highly potent anorectic substance. The intra-cerebroventricular administration of 10-20 μg satietin suppresses food intake in rats during the first day of feeding after deprivation of food for 96 hours to half of the amount eaten by untreated controls (ID_{50}). The onset of the effect can be detected within 30 minutes, the peak effect is reached within an hour. The effect lasts 24-30 hours.

Satietin acts both in intravenous and subcutaneous administration (ID_{50} = 0.5–0.75 mg/kg) in rats deprived of food for 96 hours. The peak effect is reached within an hour and lasts for 24 hours.

In contrast to the anorectic drugs in clinical use and to the endogenous anorectic substances (like cholecystokinin and calcitonin) satietin proved to be highly selective in suppressing food intake.

Since satietin is widely distributed in the world of vertebrates, its concentration in the blood is amazingly high, its site of effect is in the central nervous system and it induces satiety without having any other detectable central or peripheral effect, the hypothesis was forwarded that satietin may play the role of a rate limiting blood-borne satiety signal in the negative feed-back of food intake, i.e., serving as the essential chemical link connecting the gastrointestinal tract and the brain in the regulation of feeding.

Zusammenfassung

Satietin ist ein stark anorektisch wirksames Glykoprotein, das aus dem menschlichen Serum isoliert wurde und ein Molekulargewicht von 50 000 Dalton aufweist. Sein isoelektrischer Punkt liegt bei 7,0. Satietin enthält 10–15 % Aminosäuren und 70–75 % Kohlenhydrate. Seine biologische Aktivität widersteht der Wirkung von Proteasen und einer Hitzeinaktivierung.

Nach intrazerebroventrikulärer Gabe von 10–20 μg Satietin vermindert dieses bei Ratten, die 96 Stunden nicht gefüttert wurden, die Nahrungsaufnahme während des ersten Tages der Fütterung auf die Hälfte unbehandelter Kontrollen (ID_{50}). Die Wirkung tritt innerhalb von 30 Minuten ein und erreicht nach einer Stunde ihr Maximum. Die Wirkungsdauer beträgt 24–30 Stunden.

Satietin wirkt sowohl intravenös als auch subkutan verabreicht (ID_{50} = 0,5–0,75 mg/kg) bei Ratten, die 96 Stunden gefastet haben. Der maximale Effekt ist innerhalb einer Stunde erreicht und dauert über 24 Stunden an. Im Gegensatz zu anorektisch wirksamen Medikamenten und endogenen Substanzen wie Cholezystokinin und Kalzitonin besitzt Satietin eine hohe Selektivität auf die Nahrungsaufnahme.

Satietin wurde bei vielen Vertebraten nachgewiesen, weist eine hohe Blutkonzentration auf und entfaltet seine Wirkung auf das Appetitzentrum ohne sonstige zentral bzw. peripher venös nachweisbare Wirkungen. Es wurde daher die Hypothese aufgestellt, daß Satietin ein Blut-Sättigungssignal mit einer entscheidenden Rolle in der negativen Rückkoppelung der Nahrungsaufnahme darstellt.

Key words: satietin, regulation of feeding, anorectic glycoprotein, anorexia

Introduction

The prevalence of obesity and its recognition as a serious predisposing factor to ill-health and mortality increased attention to pharmacologic and other types of treatment, but the long-term results in 2–3 year follow-up studies were not considered to be very impressive. There is still a desperate need to provide a solution to the problem of obesity.

Phenylisopropylamine (amphetamine) was the first drug used as an antiobesity agent. Nathanson mentioned in 1937 that amphetamine reduces body weight, and the first clinical trials were performed a decade later by Harris et al. in 1947, and in 1948 by Williams and his coworkers.

As during the 1950's considerable evidence accumulated showing the significant deleterious effect of overweight on health, increasing attention has been devoted to anorexigenic agents. The excessive side effects and high abuse potential of amphetamine catalysed the search for safer compounds.

In the past decade important studies analyzed which neurotransmitter functions are determinant in the regulation of hunger and satiety.

It was demonstrated in different species that noradrenaline (Grossman, 1960) and adrenaline (Grossman, 1964) could elecit feeding response in very low doses when injected through chronic cannulas directly into the brains of satiated animals. Grossman's findings founded the idea that a noradrenergic and/or adrenergic mechanism stimulated feeding behavior. On the other hand, small doses of amphetamine which release primarily noradrenaline, strongly inhibit food intake. This speaks in favour of the major role of noradrenaline in the anorectic effect of small and medium doses of amphetamine.

There is, however, a second, serotonin-mediated satiety system in the brain. This system is facilitated by those amphetamine derivatives which are halogenated in the para- and/or meta-position and are highly selective and potent releasers of serotonin. Of the anorectics in medicinal practice fenfluramine is the typical drug which acts through this mechanism (Blundell, 1977).

The analysis of the mode of action of various anorectic drugs proved to be very useful in revealing the neurochemical basis of the physiological satiety mechanisms. Mazindol, which is the first drug in therapy lacking

the phenylisopropyl backbone, seems to be a good tool for learning more about dopamine-mediated satiety. Mazindol seems to exert its anorectic effect by activating selectively a dopaminergic mechanism (Kruk and Zarrindast, 1976).

As the anorectic drugs in medicinal practice act by releasing either catecholamines or serotonin in the brain, and because any of the biogenic amines released by an anorectic drug is involved in different functions in the brain and in the periphery, none of the anorectics inhibit food intake selectively.

The present state of anorectic drug therapy calls for new strategies in the treatment of obesity.

During the last decade a number of endogenous substances, mainly peptides, were reported to decrease food intake in animals. This new line of research started with the paper of Gibbs et al. in 1973 who described that cholecystokinin (CCK) decreases food intake in rats. Before this observation only glucagon (1957), the prostaglandins (1964) and enterogastron (1967) were claimed to possess a food intake suppressing effect. Table 1 shows the variety of endogenous substances, mostly known peptide hormones, recognized as having a food intake suppressing effect. The increased interest in this field is clearly shown by the table, as 12 of the 16 items on the list were published during the last five years.

Of the compounds listed in table 1 calcitonin is the most potent anorectic substance with the longest duration of effect. Because of its specific hormonal effect, however, its long-term use as an antiobesity drug is out of the question.

None of the endogenous substances specified in table 1 had provided a real advantage over the anorectic drugs in clinical practice.

In 1979 we described the presence of an anorectic substance, named satietin, in human serum which, in contrast to the anorectic drugs in clinical use and to the endogenous anorectic substances, proved to be highly specific in inhibiting food intake (Knoll, 1979, 1980, 1982a, b, c).

Table 1. List of endogenous anorectic substances in the chronological order of the recognition of their effect on feeding (satietin is omitted).

1957	Glucagon (Schulman et al.)
1964	PGE and $PGF_{2\alpha}$ (Horton)
1967	Enterogastron (Schally et al.)
1973	Cholecystokinin (Gibbs et al.)
1977	Thyrotropin releasing hormone (Vijayan and McCann)
	Beta-endorphin→Naloxone (Grandison and Guidotti)
	Pancreatic polypeptide (Malaisse-Lagae et al.)
1978	pGlu-His-GlyOH (Reichelt et al.)
1979	Somatostatin (Lotter et al.)
	Bombesin (Gibbs et al.)
	Calcitonin (Freed et al.)
	Insulin (Woods et al.)
1981	Vasoactive intestinal peptide (Woods et al.)
1982	Corticotropin releasing hormone (Morley and Levine)
	2-Deoxytetronate (Oomura et al.)
	Neurotensin (Hoebel et al.)

The isolation of satietin from human serum, and the chemical nature of the substance

Figure 1 shows the isolation procedure of satietin from human serum.

Satietin is a glycoprotein with a molecular weight of 50,000 dalton. Its isoelectric point is 7.0. It contains 14–15 % amino acids and 70–75 % carbohydrates. Its biological activity survives digestion with proteases (trypsin, chymotrypsin, and carboxypeptidase) and boiling (Knoll, 1979, 1980, 1982a, b, c, Nagy et al., 1982, 1983).

The homogeneity of satietin was proved by polyacrylamide gelelectrophoresis in the presence of sodium dodecyl sulfate (SDS), gradient gel electrophoresis in slab gel and analytical isoelectric focusing.

Satietin is identically stained with ninhydrin and perjodic acid-Schiff reagents indicating the glycoprotein nature of the substance.

The following amino acids, expressed in per cent, were detected in satietin:

Asp. 1.21; Thr 0.66; Ser 0.71; Glu 1.87; Pro 0.45; Gly 0.42; Ala 1.58; Cys 0.13; Val 0.55; Met 0.12; Ile 0.26; Leu 0.90; Tyr 0.31; Phe 0.44; Hys 3.79; His 0.15; Arg 0.49. Total: 14.04 %.

Mannose, galactose, glucose, and glucosamine are the detected carbohydrate components.

Freeze-dried satietin is a white, easily water-soluble and stable amorphous powder.

Satietin in the serum of different mammals and in poultry blood

Satietin was found to be present in the serum of all species of rodents, such as mouse, different strains of rats (Wistar, Long Evans, CFY, SHR), guinea pig and rabbit, which were selected for examination (Knoll, 1980).

Satietin was also detected in bovine and horse sera, which were taken as examples of the ungulates, and further in the serum of the cat and dog, representing the order of carnivora (Knoll, 1980).

As we were able to obtain a few liters of goose serum we selected this species belonging to the order of anseriformes to check the presence of satietin in avian blood. We found an anorectic substance in goose serum which was gelchromatographically indistinguishable from the active substance prepared from the sera of mammals (Knoll, 1982c).

The isolation of satietins detected in horse, cattle and goose sera is in progress. According to preliminary observations these satietins seem to be closely related glycoproteins but not identical with each other or with human serum satietin.

The anorectic effect of satietin

Satietin proved to be a highly potent anorectic substance which inhibited food intake dose-dependently in rats deprived of food for 96 hours. Table 2 shows the dose-dependent and long-lasting suppression of food intake in hungry rats by satietin isolated from human serum.

A biological assay for measuring satietin activity in units was developed. The unit is equivalent to the anorexigenic activity of the amount of a satietin sample which, when given intra-cerebroventricularly, decreases the chow pellet consumption of rats deprived of food for 96 hours during

the first day of feeding from 24.04 ± 0.76 g to 10 g. With higher intracerebroventricular doses of satietin (2–3 units/rat) the 24 h consumption of the fasting rats can be reduced to 3–5 g and the animals begin to eat on the second day of feeding only. The satietin sample the effect of which is

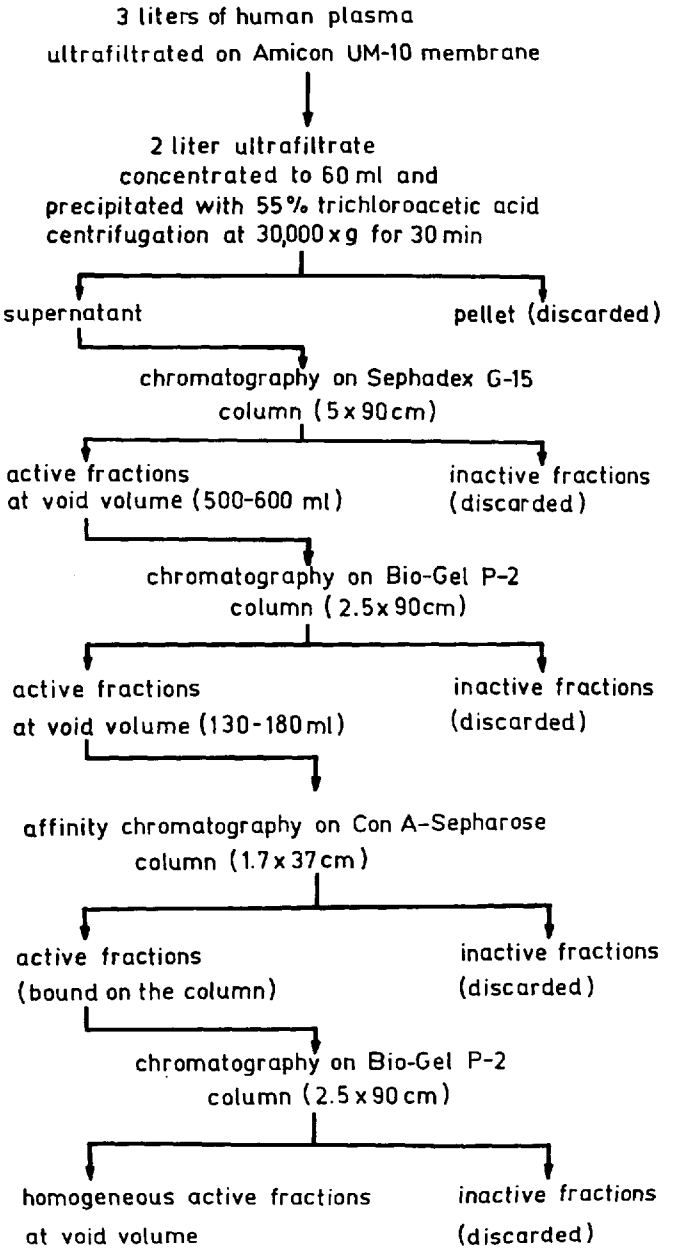

Fig. 1. Flow sheet of the isolation procedure of satietin.

Table 2. An example of the bioassay of a highly purified satietin sample by measuring its dose-dependent anorectic effect in rats deprived of food for 96 hours.

Treatment	µl/rat	Food intake mean ± S.E.M. g/1^h	g/24^h	
Saline	20	7.58 ±0.63	23.92 ±0.99	
	µg/rat			Satietin activity expressed in units
Satietin	2.5	5.58 ±0.79	19.02 ±0.87	0.25
	5	4.90* ±0.68	13.18 ±0.69	0.50
	7.5	3.88* ±0.92	12.15* ±1.19	0.75
	10	1.88** ±0.41	9.82** ±0.87	1.00
	20	0.62** ±0.28	6.51** ±0.94	2.00
	40	0.78** ±0.34	3.22** ±1.29	4.00

Rats were deprived of food for 96 hours. Water was supplied ad libitum.
The satietin sample was prepared from human serum. Satietin dissolved in saline was injected in a volume of 20 µl/rat intra-cerebroventricularly into the lateral ventricle one hour before feeding. Each dose was tested on 16 rats. The consumption of chow pellets (g) during the first hour of feeding (g/1^h) and for 24 hours (g/24^h) is shown in the table.
Statistics: Student's t-test for two means
* $p < 0.05$, ** $p < 0.001$

shown in table 2 contained, according to the definition of the arbitrary unit, 100 units/mg activity.

Satietin exerts a dose-dependent anorectic effect in rats both on intravenous and on subcutaneous administration. The intra-cerebroventricular administration of 10 µg satietin suppresses food intake in rats during the first day of feeding after deprivation of food for 96 hours to half of the amount eaten by untreated controls (ID_{50}). The onset of the effect can be detected within 30 minutes, the peak effect is reached within an hour. The effect lasts 24–30 hours. Following intravenous administration, the ID_{50} of satietin was found to be 0.5–0.75 mg/kg in rats deprived of food for 96 hours. The peak effect was reached within an hour and lasted for 24 hours.

Most of the biological information on satietin stems from intra-cerebroventricular administration of the substance. The difficulties involved in the isolation of satietin have so far restricted the parenteral administration of highly purified material and permit virtually only the intra-cere-

Table 3. The unchanged efficiency of two consecutive doses of satietin and the lack of rebound in normally fed rats.

Days	1	2	3	4	5	6	7	8	9	10	11	12
Daily food consumption (g)	26.2	6.89*	14.8*	27.4	25.2	24.2	4.5*	13.2*	21.8	26.6	29.2	27.4
	↑ satietin					↑ satietin						

* significant $p < 0.001$
Statistics: Student's t-test for two means
n = 12; 40 µg satietin was injected intra-cerebroventricularly (right and left side)

broventricular application of pure satietin. For the same reasons the long-term anorectic efficiency of satietin also remains to be proved.

Table 3 shows the unchanged efficiency of two consecutive doses of homogeneous satietin in a group of normally fed rats. The effect of the first dose of satietin lasted about 48 hours and the food consumption returned to normal on the 3rd day after the intra-cerebroventricular injection of satietin. There was no sign of rebound. The intensity and the time course of effect of the second dose of satietin were identical with that of the first dose. Food consumption was severely depressed for 48 hours after the injection of satietin, but no significant difference in the 3rd day's consumption was to be observed. Food consumtion again remained normal on the consecutive days.

These experiments induce the hope that in spite of the long-lasting anorectic effect of satietin, the continuous administration of the substance will not change the onset and offset of its anorectic effect and it will not interfere with the normal endogenous regulation of food intake. To find out, however, if this hope is well-grounded, an abundant supply of satietin is needed to perform the necessary long-term experiments.

The food intake suppressing effect of satietin in comparison with endogenous anorectic substances

Endogenous substances with anorectic effect were compiled in table 1. Calcitonin is the only endogenous substance which needs careful analysis in relation to satietin, as it is as potent as satietin in suppressing food intake in rats deprived of food for 96 hours, whereas all other endogenous substances reported to have anorectic effect are ineffective in animals with such an intensive hunger drive. Calcitonin has its specific hormonal effect and it inhibits bone resorption by altering osteoclastic and osteocytic activity. Considering its high potency in influencing calcium metabolism, which is in harmony with the very low physiological concentrations (70–120 picogram/ml) of this hormone, it inhibits food intake in relatively high amounts only. The reduction of feeding in rats by calcitonin is evidently secondary to its inhibition of calcium uptake into hypothalamic nerves, as was shown recently by Levine and Morley (1981). Even if it lacks the selectivity of satietin, its potential role as a blood-borne satiety signal needs consideration. If we take into account that the satietin concentration in human serum is over 2 µg/ml, the difference on a weight basis is about 20,000 and on a molecular basis (50,000 versus 3,600) about 1,500. The intra-cerebroventricular efficacy of calcitonin on a weight basis is about 10 times higher than that of satietin, but satietin is a 13.88 times bigger molecule. Thus, the two peptides can be taken to be roughly equally active as anorectic agents.

Comparing the anorectic activities and the blood concentrations of satietin and calcitonin one can hardly believe that calcitonin takes a prominent part in the physiological regulation of feeding as a blood-borne satiety signal when we have in our blood an anorexigenic glycoprotein, satietin, in a molar concentration at least 1,500 times higher, which is at least as potent as calcitonin in suppressing food intake and is in addition much more selective. Thus calcitonin's effect as a blood-signal must be

negligible compared with that of satietin. However, as calcitonin is also released in the brain, it might be involved in the physiological regulation of feeding as a locally acting hormone in the hypothalamus.

The food intake suppressing effect of satietin in comparison with anorectic drugs

The anorectic effect of satietin is different from any type of the anorectic drugs. The first important difference is in the duration of the effect. Single doses of amphetamine, fenfluramine and mazindol have no significant influence on the 24-hour consumption in rats after 96 hours of food deprivation because their anorectic effect lasts only 4–5 hours.

The anorectic effect of amphetamine and mazindol which act via the release of catecholamines is considerably weakened in rats pretreated with alpha-methylparatyrosine (alpha-MT), which inhibits the synthesis of the catecholamines whereas the effect of satietin remains unaltered in the alpha-MT pretreated animals (Knoll, 1979, 1980, 1982a).

The anorectic effect of fenfluramine was prevented by lesioning the median and dorsal raphe (Knoll, B. et al., 1982), whereas the effect of satietin remained unaltered in the lesioned rats.

The time course of the effect of satietin in comparison with that of amphetamine, fenfluramine and calcitonin was studied in an eatometer. Blundell found differences in the anorectic effect of amphetamine and fenfluramine by analysing the microstructure of eating, and concluded that amphetamine which activates catecholaminergic transmission acts as a suppressor of the onset of eating, whereas fenfluramine facilitates satiety (for review see Blundell and Latham, 1982).

By measuring the time from the beginning of the experiment to the consumption of the first bite in rats following 24-hour starvation, we were able to corroborate the finding of Blundell. Amphetamine (1 mg/kg, s.c.) significantly extended the latency time of the beginning of eating, whereas fenfluramine (3 mg/kg, s.c.), satietin (4 mg/kg, s.c.) and calcitonin (10 MRC units/kg, s.c.) all injected 30 minutes before feeding, did not change the onset of eating (Knoll, 1982b, 1982c, Sándor and Knoll, to be published).

Figure 2 shows the typical curves for fenfluramine and amphetamine. The two doses were selected to be equipotent in inhibiting the total consumption of food during one hour of feeding in rats deprived of food for 24 hours. It is evident from this figure that fenfluramine is less active in inhibiting food intake in the first 15 minutes than amphetamine. The amphetamine-treated rats eat equally small amounts of food during the four consecutive quarters of the one-hour feeding period, while fenfluramine blocks eating completely during the second half of the feeding period. Figure 3 shows that both satietin and calcitonin act similarly to fenfluramine, neither being very active in inhibiting food consumption during the first 15-min period of feeding, but later on a complete suppression of feeding was to be observed. For comparison the time structure of food intake under the influence of amphetamine is also shown in the figure.

These experiments speak in favour of the assumption that satietin acts by facilitating satiety. In this respect fenfluramine acts similarly, but there is an important difference in the mechanism of action between the two

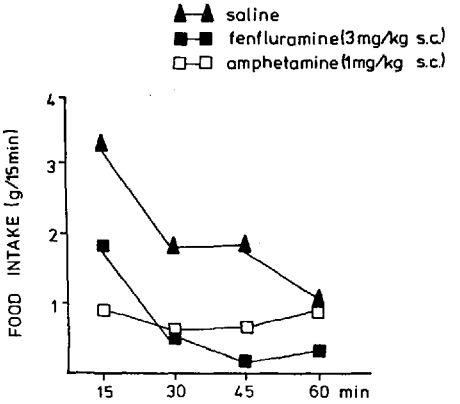

Fig. 2. The time course of the effect of fenfluramine and amphetamine in comparison to saline-treated rats deprived of food for 24 hours. Measurements were performed in an eatometer. Both substances were administered subcutaneously 30 min before feeding.

substances, as the anorectic effect of fenfluramine is prevented by the lesioning of the raphe system, whereas that of satietin remains unaltered in the lesioned animals.

To illustrate the anorectic potency of satietin in comparison with anorectic drugs, the relative potency of satietin to fenfluramine in rats deprived of food for 96 hours was evaluated. In comparison with satietin, fenfluramine is short acting. Only the consumptions in the first hour are comparable. The compounds were administered intra-cerebroventricularly 1 hour before feeding. About 50 µg fenfluramine was found to be equivalent to 20 µg satietin. Considering the molecular weights (50,000 versus 181) satietin proved to be 690 times more potent than fenfluramine

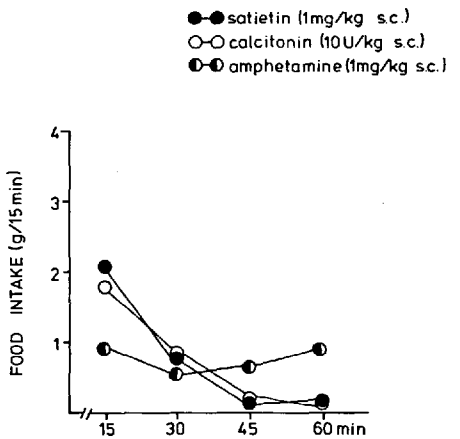

Fig. 3. The time course of the effect of satietin in comparison to that of calcitonin and amphetamine in rats deprived of food for 24 hours. Substances were administered subcutaneously 30 min before feeding.

in suppressing food intake in hungry rats when given intra-cerebroventricularly. By comparing the relative activities at intravenous administration we found that, on a molecular basis, satietin was at least 500 times more potent than fenfluramine in inhibiting the first-hour consumption of food in rats deprived of food for 96 hours.

The selectivity of the anorectic effect of satietin

The anorectic drugs in medicinal practice act by releasing either catecholamines or serotonin, and because any of the biogenic amines released by an anorectic drug is involved in different functions in the brain and in the periphery, none of the anorectics inhibit food intake selectively. The lack of selectivity leads to a number of side effects and anorectic drugs are mainly considered to be short-term adjuvants in a more complex therapy of obesity including calorie restriction, appropriate exercise and psychological support.

The potential medicinal use of any of the endogenous substances listed in table 1 as anorectic agents is out of the question, as all of them have characteristic physiological activities exerted in much lower than anorectic concentrations.

Satietin seems to be at present the only known endogenous substance which does not exert any noticeable central or peripheral effect in the anorectic dose range.

Table 4 summarizes the effects of equianorectic doses of satietin, calcitonin, amphetamine and fenfluramine on the behavior of rats in a battery of tests. Fenfluramine, in the anorectic dose, was found to be a strong inhibitor in all the tests studied. It decreased locomotion in the open field, strongly interfered with unconditioned avoidance reactions, inhibited the development of conditioned reflexes, blocked learning and retention in one-way and two-way avoidance systems, inhibited the recall of a previously firmly developed conditioned response and completely inhibited copulatory behavior in male rats. In contrast to fenfluramine, amphetamine was stimulatory in the tests and facilitated performances. The anorectic dose of calcitonin inhibited the acquisition of a conditioned reflex in a one-way avoidance system and blocked the recall of a firmly established conditioned response, however, in three other tests it left the rat's performances unchanged. Satietin was ineffective in all tests.

Table 5 compares the effects of satietin, calcitonin, amphetamine and fenfluramine on the metabolic rate, body temperature and blood pressure. Again, none of these parameters were influenced by a dose of satietin which blocked food intake in the rat completely.

That satietin is highly selective in suppressing food consumption is further supported by the finding that the intra-cerebroventricular administration of 1-2 units of satietin into the lateral ventricle, which exerts a strong anorectic effect, has no effect on the water intake of rats. Figure 4 shows an example. Groups of rats were deprived of water for 23 h and injected intra-cerebroventricularly 1 h before water supply with satietin (0.25, 0.5, 1 and 2 U) and calcitonin (0.125, 0.25, 0.5 and 1 U), respectively. The control group was treated with saline, 20 µl/animal. Water was given at the end of the 24-h deprivation period and its consump-

Table 4. Comparison of the effect of satietin, calcitonin, amphetamine and fenfluramine on the behavior of rats in a battery of tests.

	Number of rats	Satietin i.c.v.	Calcitonin i.c.v.	Amphetamine i.v.	Fenfluramine i.c.v.	Method
Locomotor activity	10	none	none	strong facilitation	strong inhibition	open field
One-way avoidance	10	none	none	strong facilitation	strong inhibition	modified jumping test (Knoll and Knoll, 1964)
Two-way avoidance	12	none	none	facilitation	inhibition	shuttle-box
One-way conditioning	10	none	strong inhibition	strong facilitation	strong inhibition	screening test I (Knoll B. et. al. 1974)
Consolidated conditioned reflex	6	none	inhibition	none	strong inhibition	jumping test (Knoll and Knoll, 1958, 1959)
Male copulatory behavior	13	none	–	facilitation	inhibition	Beach, 1944

All compounds were administered in the dose equianorectic with satietin (usually 1–2 units) either intra-cerebroventricularly (i.c.v.) or intravenously (i.v.) to a group of rats. Satietin was isolated from human serum. For methodological and other details see Knoll and Knoll, 1982, and Yen et al., 1982.

Table 5. Comparison of the effect of satietin, calcitonin, amphetamine and fenfluramine on metabolic rate, body temperature and blood pressure in the rat.

	Satietin	Calcitonin	Amphetamine	Fenfluramine	Method
Metabolic rate	none	significant increase (38%)	significant increase (97%)	none	Issekutz and Issekutz, 1942
Body temperature	none	slight, statistically insignificant elevation	slight, statistically insignificant elevation	none	continuous measurement of rectal temperature
Blood pressure	none	none	significant increase	slight decrease	via the carotid artery

All compounds were administered intra-cerebroventricularly in doses equianorectic with the dose of satietin. Satietin prepared from human serum was used in the experiments. For methodological and other details see Timár and Knoll, 1982.

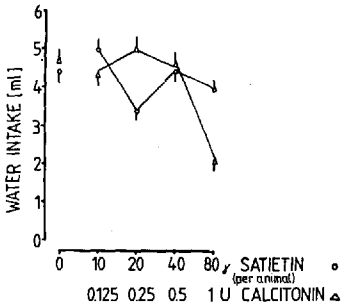

Fig. 4. Effect of satietin and calcitonin on the water intake of rats in the first 4 hours after 23 h water deprivation. 7 rats are in each group. Satietin and calcitonin were injected into the lateral ventricle in the 22nd hour of water deprivation.

tion was measured for 4 h. The animals were starving during the experiment. None of the satietin doses caused any significant change compared to the controls, while 1 MRC unit of calcitonin decreased water intake significantly ($p < 0.01$).

Satietin did not change the water intake in rats deprived of food for 96 hours and supplied with water ad libitum, as shown in table 6. It can be seen from the data in table 6 that rats fed with standard chow pellets need, because of the dry food, a high amount of water for consuming the pellets. During the 96-h starvation period the water consumption of the rats decreases to their essential need, which is about 6–8 ml daily. In contrast to satietin, amphetamine (5 mg/kg subcutaneously every 5 hour) doubled the water intake of rats (12.38 ± 1.92 ml versus 6.45 ± 0.83 ml) under the same experimental circumstances.

We have also demonstrated, by using the 'conditioned aversion' paradigm of Garcia et al. (1974), that satietin, even in higher than anorectic dose, did not elicit aversion to food (Sándor and Knoll, 1982), i.e., it is a real anorectic agent.

Table 6. Ineffectiveness of satietin on the water intake of rats deprived of food for 96 h. N = 20. Intra-cerebroventricular administration.

Treatment	Dose	Water intake (ml) ± S.E.M.					
		Before starvation	After starvation (days)				After feeding
			1.	2.	3.	4.	
Without	–	37.4 ±1.66	13.75 ±2.0	12.95 ±1.18	8.35 ±0.79	6.45 ±0.83	35.9 ±2.79
Saline*	20μ/ animal	32.28 ±1.08	15.7 ±2.12	10.0 ±0.89	7.0 ±0.63	6.6 ±0.94	38.9 ±1.15
Satietin*	80 μg/ animal	33.75 ±1.48	13.05 ±1.31	12.1 ±1.18	9.6 ±0.89	6.2 ±0.88	37.8 ±1.29

* Injected in the morning of the 4th day's starvation.

The intra-cerebroventricular administration of satietin did not change the blood sugar, insulin and glucagon levels in the blood either in normally fed rats or in rats deprived of food for 96 hours. Amphetamine, given intra-cerebroventricularly, did not change the measured parameters in normally fed animals but significantly increased the serum insulin level in food-deprived rats (Gyarmati et al., 1982).

Amphetamine, which is a potent releaser of noradrenaline, exerts a number of peripheral effects and is highly potent on isolated organs possessing noradrenergic transmission machinery. We checked the effect of satietin on the perfused central ear artery of the rabbit, on the pulmonary artery strip of the rabbit and on the isolated nictitating membrane of the cat, in which less than 1 μg/ml amphetamine strongly potentiates the response of the vascular smooth muscle to nerve stimulation. Even very high doses of satietin proved to be completely ineffective in these tests (Knoll, 1982b, 1982c).

We tested the effect of satietin in acutely spinalized rats by measuring the contraction of the m. tibialis anterior to hind paw stimulation. Serotonin is known to be involved in the spinal reflex, and fenfluramine in i.v. doses, even lower than the anorectic dose level, increases the contractions of the m. tibialis anterior to stimulation by enhancing the activity of the serotonergic link in the reflex. Satietin proved to be completely ineffective even when given intravenously in a much higher than anorectic dose (Knoll, 1982b, c).

The effect of satietin was also investigated in isolated organs (longitudinal muscle strip of the guinea pig ileum, mouse vas deferens and cat splenic strip) which are used for testing opiate agonists and antagonists. Satietin did not exert any effect in these tests and failed to influence the effect of opiate agonists.

The putative role of satietin in the regulation of food intake

Putting all the data together, the working hypothesis visualized in figure 5 is forwarded.

Considering that satietin is widely distributed in the world of vertebrates, that its concentration in the blood is amazingly high, its site of effect is in the central nervous system and that it induces satiety without having any other detectable central and peripheral effect, the hypothesis is forwarded that *it may play the role of a rate-limiting, blood-borne, satiety signal in the negative feed-back of food intake, i.e., serving as the essential chemical link connecting the gastrointestinal tract and the brain in the regulation of feeding.*

The amazing selectivity of satietin might be explained by the assumption that neurons exist in the brain with highly specific satietin receptors and function as a 'satiety center'. If, because of the high satietin concentration in the blood, these receptors are saturated with satietin, food intake is completely inhibited, and we have the subjective feeling of fullness, and possibly even an aversion for food. Satietin might be liberated from inactive binding by special signals. Regulator(s) of the liberation of physiologically active satietin may play important roles in satiation and there

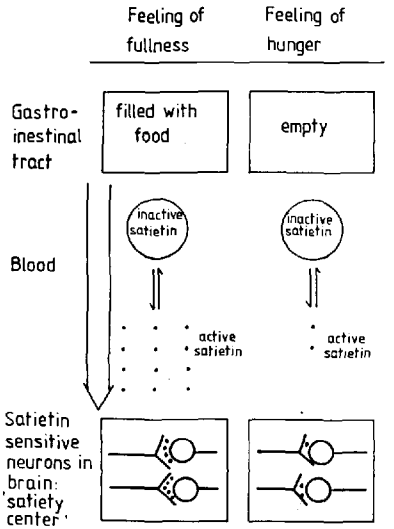

Fig. 5. Scheme visualizing the hypothesis that, satietin, a blood-borne substance, plays the role of a rate-limiting satiety signal in the negative feed-back of food intake.

is also a place for short term satiety signal(s) originating from the gastrointestinal tract.

That satietin circulates in the blood in an inactive form is strongly supported by the finding that, whereas with the material prepared by the method without the step of trichloroacetic precipitation (Knoll, 1982c), the peak effect was reached 5 h after the intra-cerebroventricular injection of the sample, the trichloroacetic acid precipitation of albumin before gel chromatographic separation, see figure 1, yielded preparations with a short (30 min) onset of the effect.

Digestion of food and the emptying of the gastrointestinal tract may lead to a gradual decrease of active satietin in the blood, thus the satietin concentration in the brain, too, is step by step decreasing, leading to the inactivation of the 'satiety center' and to the progressively intensive feeling of hunger as visualized in figure 5.

If satietin were the blood-borne rate-limiting satiety signal in the negative feed-back of food intake this would have eagerly expected theoretical and practical consequences. Theoretically it would mean a decisive step forward in the elucidation of the biogrammar of feeding. From a practical point of view, an unhoped for new opportunity had presented itself for influencing food intake. According to the hypothesis, hunger is terminated by the liberation of physiologically active satietin in the blood and eating an appropriate amount of food leads to this change. As the brain should be satiated by satietin, if we could learn to raise, to a sufficient extent, the blood level of the satiety signal by exogenous manipulation, we could terminate the feeling of hunger by avoiding the natural route: filling the gastrointestinal tract. We may thus have the option to terminate hunger drive either by the natural way, eating, or artificially by increasing

the satietin concentration in the blood exogenously. To get to the bottom of this possibility is one of the main subjects of our research.

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Author's address:

Prof. J. Knoll, Department of Pharmacology, Semmelweis University of Medicine, Budapest, Nagyvárad tér 4, H-1089, Hungary