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ORIGINAL CONTRIBUTION

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Circadian anorectic effects of peripherally administered amylin in rats

Zirkadiane anorektische Effekte von peripher verabreichtem Amylin bei Ratten

Summary The pancreatic peptide amylin (1 μg/kg) injected intraperitoneally reduced cumulative food intake for up to 4 h in food-deprived (24 h) and non-deprived rats at various times of the day, i.e., at dark onset, in the middle of the dark phase, and at light onset. At none of these times did subdiaphragmatic vagotomy abolish the anorectic effect of amylin. Rather, vagotomy enhanced, by unknown mechanisms, amylin's anorectic effect in food-deprived rats at light onset and in the middle of the dark phase. In contrast to previous studies with older rats, amylin's anorectic effect was also observed when injected into nondeprived rats. The findings of the present study extend previous reports in that amylin's anorectic effect, not being abolished by abdominal vagotomy after intraperitoneal injection, can be elicited at different times of the day.

Zusammenfassung Das aus dem Pankreas stammende Peptid Amylin (1 μg/kg) reduzierte nach intraperitonealer (IP) Injektion die kumulative Futteraufnahme bei Ratten, denen vor der Injektion das Futter für 24 h entzogen worden war bzw. bei Ratten, denen Futter ad lib. zur Verfügung stand, für bis zu 4 h. Der Effekt trat zu verschiedenen Zeitpunkten des Tag/Nacht-Zyklus (Beginn der Dunkelphase, Mitte der Dunkelphase, Beginn der Hellphase) auf. Der verzehrsreduzierende Effekt von IP verabreichtem Amylin wurde zu keinem dieser Zeitpunkte durch subdiaphragmatische Vagotomie aufgehoben. Vagotomie führte eher zu einer Verstärkung des verzehrsreduzierenden Effekts von Amylin, und zwar nach einem 24stündigen Futterentzug bei Injektion zu Beginn der Hellphase oder in der Mitte der Dunkelphase. Im Unterschied zu früheren Untersuchungen mit älteren Ratten konnte ein verzehrsreduzierender Effekt von Amylin auch bei Ratten beobachtet werden, denen vor der Injektion das Futter nicht entzogen worden war.

In Ergänzung früherer Befunde zeigen also die Ergebnisse, daß der verzehrsreduzierende Effekt von IP verabreichtem Amylin zu verschiedenen Tageszeiten ausgelöst und durch subdiaphragmatische Vagotomie nicht aufgehoben werden kann.

Key words Amylin – food intake – vagotomy – circadian effect – rat

Schlüsselwörter Amylin – Futteraufnahme – Vagotomie – zirkadiane Effekte – Ratte

Abbreviation index CGRP = calcitonin gene-related peptide · *IP* = intraperitoneal · *SVAG* = sham vagotomized · *VAG* = vagotomized

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Introduction

The main constituent of pancreatic amyloid deposits of human insulinoma, type 2 diabetics, and diabetic cats is the pancreatic peptide amylin, or islet amyloid polypeptide (37, 38). Since its discovery in 1986 amylin has been shown to be a normally occurring secretory product of pancreatic B cells in many species rather than a peptide specifically synthesized during the mentioned disorders (14, 38). However, amylin has been implicated in the pathogenesis of human type 2 and feline diabetes mellitus due to its propensity to precipitate as amyloid in these

species, which may lead to secondary B cell degeneration, and due to its main effects on glucose metabolism, which are an inhibition of insulin secretion and the induction of peripheral insulin resistance (3, 19, 34, 35, 40, 41).

Amylin consists of 37 amino acids and is structurally related to calcitonin gene-related peptide (CGRP) which is widely distributed throughout the peripheral and central nervous system (for review (13, 29)). Both peptides have a disulfide bridge between the cysteine residues at positions 2 and 7 and are biologically active in their amidated form (9). Amylin is coreleased with insulin in response to appropriate stimuli, for example, an elevation in blood glucose concentration and meal ingestion (5, 22, 27). The plasma concentration of CGRP also increases after food intake, but the mechanism of the meal-induced rise in plasma CGRP remains unclear (43). In contrast to amylin, the main source of plasma CGRP seems to be CGRP leaking from nerval tissue into the circulation rather than endocrine cells (11, 42).

Previous studies have shown that both amylin and CGRP elicit an anorectic response if administered centrally (intracerebroventricularly or intrahypothalamically) or peripherally in mice and rats (1, 6, 7, 8, 16, 17, 20, 24-26, 33). In earlier studies we have shown that an anorectic effect of amylin injected intraperitoneally (IP) can be elicited in food-deprived rats at low amylin doses with a minimal effective dose of 0.5 μg/kg (20). The anorectic effect of amylin at doses equal to or above 2.5 μg/kg was not more pronounced than at 1 μg/kg (20). It has been shown that an anorectic effect of amylin can be elicited in rats fed a medium-fat, a carbohydrate-free high-fat, or a high-carbohydrate low-fat diet (20, 21), and in rats of different age when investigated under appropriate conditions (20, 25).

At dark onset IP injected amylin reduces food intake in food-deprived rats independently of an intact abdominal vagus and has no influence on gastric emptying (21). This is in contrast to CGRP whose anorectic effect seems to depend partly on slowing gastric emptying (18, 28, 30).

The effects of several peptides on feeding vary according to the time of administration. Neuropeptide Y, for example, which has been shown to increase selectively the intake of carbohydrates, is most effective at dark onset (for review (39)). The anorectic effect of glucagon also seems to differ during the diurnal light/dark cycle and is furthermore differentially influenced by hepatic branch vagotomy (12, 36). Similarly, the feeding response induced by IP administration of the glucose antimetabolite 2-deoxy-D-glucose seems to depend on the common hepatic vagus branch only at certain times of the day (10).

The present study therefore investigated whether the efficacy of amylin in reducing food intake depends on the time of administration. Part of the experiments were performed using subdiaphragmatically vagotomized rats to examine whether the influence of vagotomy on amylin's effect on food intake depends on the time of day. Amylin (1 μg/kg) was administered IP at dark onset, in the middle of the dark phase, and at light onset in fasted rats or without prior food deprivation.

Materials and methods

Adult male rats (ZUR:SIV rats, Institut für Labortierkunde, University of Zürich, Switzerland) with a mean body weight of approximately 250 g (intact rats), 400–500 g (subdiaphragmatically vagotomized (VAG) rats), or 500–600 g (sham vagotomized (SVAG) rats) were used for the experiments.

The rats were adapted to the laboratory conditions for at least 4 weeks and were housed in individual wire cages in a controlled environment (room temperature: 21° ± 1°C; 12-h light-dark cycle, lights on 0800 hours). Intact rats were fed a powdered medium-fat diet (MF; Kliba Mühlen, Kaiseraugst, Switzerland) with a fat content of approximately 18 % and 46 % carbohydrate. VAG and SVAG rats were fed an isoenergetic high-carbohydrate low-fat diet (HC) with approx. 3 % fat and 77 % carbohydrate (21). (Table 1)

Table 1 Composition of medium-fat medium-carbohydrate (MF) and high-carbohydrate low-fat (HC) diets (percentages)

	MF	HC
Casein[a]	12.87	12.87
Corn starch[b]	46.00	76.67
Soy-bean oil[c]	3.47	3.33
Beef tallow[d]	9.42	–
Lard[d]	5.17	–
Vitamin premix[e]	3.00	3.00
Mineral premix[e]	4.00	4.00
Methionine[a]	0.13	0.13
Polyethylene powder[f]	16.00	–

[a] UFAG, Sursee, Switzerland; mean protein content 89 %
[b] Blattman, Wädenswil, Switzerland
[c] Sais, Horn, Switzerland
[d] Häute- und Fettwerk, Zürich, Switzerland
[e] Kliba Mühlen, Kaiseraugst, Switzerland
[f] BASF, Ludwigshafen, Germany

Rat amylin was obtained from Peninsula Laboratories (Belmont CA, USA). Amylin was dissolved in 0.9 % saline solution and injected IP at a dose of 1 μg/kg body weight. The injection volume was 1 ml/kg, injection of saline served as control.

Amylin was injected in nondeprived intact rats at dark onset (2000 hours; 1 μg/kg). In VAG and SVAG rats, respectively, amylin was injected after 24 h of food deprivation at light onset (0800 hours; 1 μg/kg) and after

24 h of food deprivation or without deprivation in the middle of the dark phase (0200 hours; 1 µg/kg). Food intake was determined by manually weighing the food containers and correcting for spillage. Cumulative food intake was measured 0.5, 1, 2, 4, 6, and 12 h after amylin or saline injection. In all experiments water was available ad libitum.

On the day before the experiments food intake was measured in all rats to be grouped accordingly to yield experimental and control groups (within intact, or VAG and SVAG rats, respectively) with similar food intake and body weight.

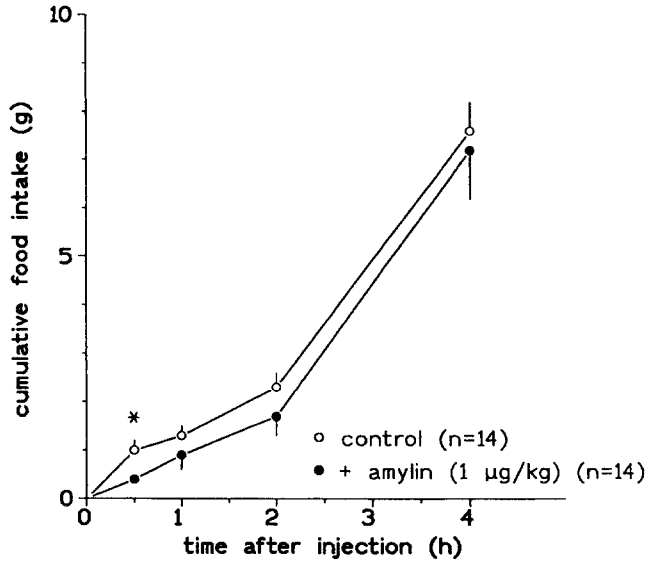
Subdiaphragmatic vagotomy

Bilateral dissection of the abdominal vagus was performed as described earlier (21). Briefly, rats were anesthetized with ketamine hydrochloride (66 mg/kg body weight) and xylazine (8 mg/kg). After midline laparotomy the upper abdominal organs were exposed and the esophagus with adjacent anterior and posterior vagal trunk identified. Both trunks were ligated immediately subdiaphragmatically, i.e., proximal to the branching off of the common hepatic vagus branch, with two silk ligatures each and severed in between. SVAG rats undergoing the same procedure except for ligation and transsection of the trunks served as controls. Successful total subdiaphragmatic vagotomy was confirmed by the demonstration of a drinking deficit in VAG rats in response to hyperosmotic saline injection (21, 31, 32) and the demonstration of an attenuation of cholecystokinin's anorectic effect by vagotomy (15, 21, 23).

Statistics

Results are presented as means ± standard error of the mean. Treatment groups were compared using unpaired Student's t test (intact rats). Analysis of variance with the paired Student's t test as post hoc test for paired observations within VAG and SVAG groups or two-factor analysis of variance (effect of amylin treatment; effect of vagotomy) were used to analyze the influence of vagotomy on amylin's anorectic effect. A P value < 0.05 was considered significant.

Fig. 1 Influence of amylin (1 µg/kg) on cumulative food in nondeprived young adult rats injected at dark onset ($n = 14$). * $P < 0.05$, amylin-treated vs. control rats (unpaired Student's t test)

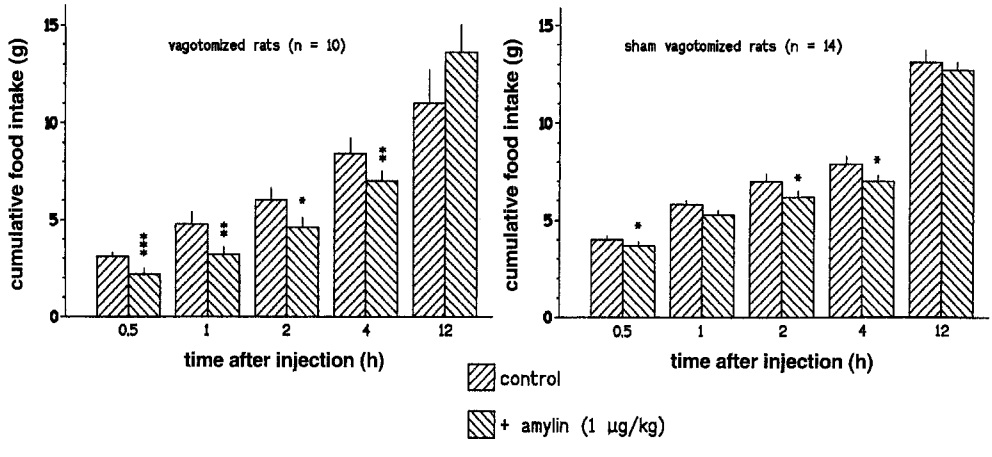


Results

First, the effect of an IP injection of amylin (1 µg/kg) at dark onset was investigated in nondeprived young adult rats. Amylin significantly reduced cumulative food intake 30 min after injection (Fig. 1). The difference in cumulative food intake between amylin-treated and control rats was maintained throughout the observation period (4 h) but did not reach the level of significance 1, 2, or 4 h after injection.

In a preliminary experiment we have shown that amylin (1 µg/kg) administered at dark onset in 12-h food-

Fig. 2 Influence of amylin (1 µg/kg) on cumulative food intake in 24-h food-deprived VAG ($n = 10$) and SVAG ($n = 14$) rats injected at light onset. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, amylin-treated rats vs. respective controls (post hoc paired Student's t test)



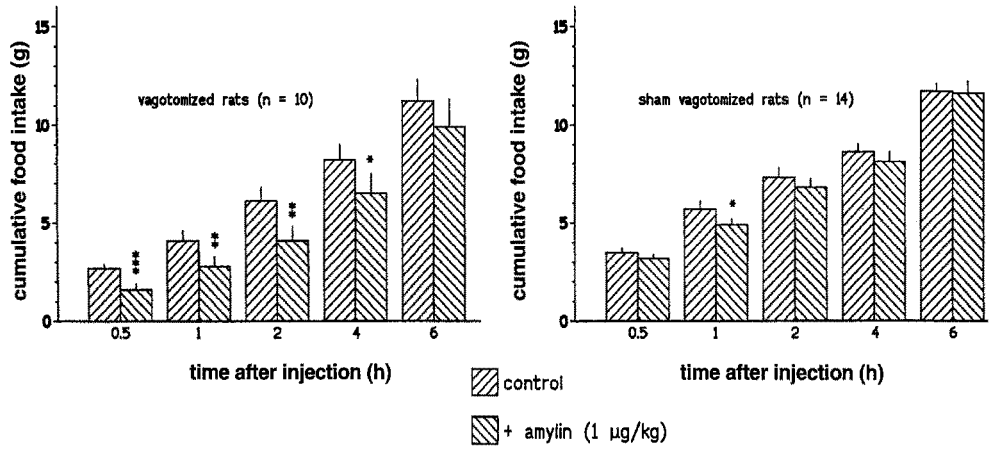


Fig. 3 Influence of amylin (1 µg/kg) on cumulative food intake in 24-h food-deprived VAG (n = 10) and SVAG (n = 14) rats injected in the middle of the dark phase. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, amylin-treated rats vs. respective controls (post hoc paired Student's t test)

deprived intact rats fed the HC diet used in the experiments with VAG and SVAG rats reduces cumulative food intake for 1 h after injection (t = 30 min: 2.6 ± 0.2 g in controls vs. 2.1 ± 0.1 g in amylin-treated rats, $P < 0.05$, paired Student's t test, n = 14; t = 1 h: 3.5 ± 0.2 vs. 2.5 ± 0.1, $P < 0.001$).

Amylin (1 µg/kg) injected at light onset after 24-h food deprivation significantly reduced food intake in both VAG and SVAG rats fed the HC diet (Fig. 2). Within the VAG or SVAG groups, the reduction in cumulative food intake in amylin-treated compared to control animals was significant for 4 h. This was confirmed by two-factor analysis of variance in which the overall effect of amylin on food intake was significant for 4 h. Furthermore, vagotomy seemed slightly to enhance amylin's anorectic effect 30 min and 1 h after injection, although this effect of vagotomy was not statistically significant (two-factor interaction: P = 0.15 after 30 min, P = 0.08 after 1 h). Twelve hours after injection the anorectic effect of amylin was compensated.

When injected in the middle of the dark phase, amylin (1 µg/kg) significantly reduced cumulative food intake in 24-h food-deprived VAG rats for 4 h (Fig. 3). In SVAG rats food intake was significantly diminished only 1 h after injection (Fig. 3). The overall effect of amylin was significant for 2 h after injection (two-factor analysis of variance) and, as in the previous experiment, vagotomy did not abolish but rather seemed to enhance amylin's anorectic effect (two-factor interaction: P = 0.08 at t = 30 min, P = 0.15 at t = 2 h).

Only a weak anorectic effect of amylin in VAG and SVAG rats was observed when IP injected in the middle of the dark period without prior food deprivation (Fig. 4). The reduction in cumulative food intake by amylin was statistically significant only in SVAG rats 4 h after injection ($P < 0.05$). Two-factor analysis of variance indicated that amylin's anorectic affect is basically uninfluenced by vagotomy (t = 4 h: $P < 0.05$ for effect of amylin, P = 0.50 for effect of interaction of amylin and vagotomy).

Discussion

Three major findings were observed in the present study: (a) the anorectic effect of IP amylin occurs at various times of the day; (b) the anorectic effect of IP amylin is not abolished or attenuated by transsection of the abdomi-

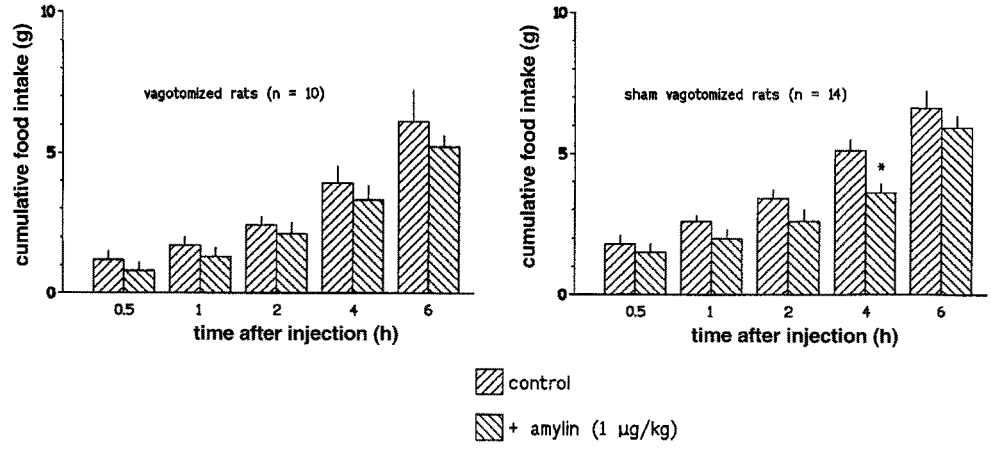


Fig. 4 Influence of amylin (1 µg/kg) on cumulative food intake in nondeprived VAG (n = 10) and SVAG (n = 14) rats injected in the middle of the dark phase. * $P < 0.05$, amylin-treated rats vs. respective controls (post hoc paired Student's t test

nal vagus at any time of day; and (c) amylin's anorectic effect can also be observed after IP injection in nondeprived rats.

The present study shows that amylin administered IP at a low dose in food-deprived rats elicits an anorectic effect of similar strength when administered at light onset or in the middle of the dark phase. At all times the anorectic effect of amylin was observed to last for a maximal duration of 4 h after injection. In contrast to the orexigenic neuropeptide Y which appears to be most effective at dark onset when rats naturally ingest most of their food with a preference for carbohydrate (39), the acute IP administration of amylin therefore seems to elicit an anorectic effect independently of the diurnal feeding cycle.

The second main result of this study is that total abdominal vagotomy does not disrupt the anorectic effect of amylin administered IP at various times of the day. After food deprivation there was rather a tendency for vagotomy to enhance the anorectic effect of amylin at light onset and in the middle of the dark phase. The same was not observed with amylin injected in nondeprived rats in the middle of the dark phase (present study) or after food deprivation at dark onset (21). The reason for abdominal vagotomy to enhance rather than to reduce or abolish amylin's anorectic effect under certain conditions remains to be investigated in future studies. Morley and coworkers (26) recently also observed that vagotomy in mice appears to increase the anorectic effect of a high dose of amylin (100 μg/kg IP).

The fact that amylin's anorectic effect is not disrupted by abdominal vagotomy supports the view that IP administered amylin has a central site of action (21), although it cannot be excluded that, other than vagal, afferent fibers originating in the abdomen are responsible for the transmission of amylin's effect. Amylin whose secretion from the pancreas is increased in response to food intake (5) may gain access to the central nervous system after crossing the blood brain barrier (21) or may act on central sites devoid of a blood brain barrier. Central amylin receptors (2, 4) may then mediate the anorectic effect of IP amylin by interacting with the dopamine or serotonin feeding regulating systems (6). As amylin reduces food intake independently of the diet composition ((20, 21) and this study), it seems rather unlikely that amylin affects food intake indirectly via its main metabolic effects (reduction in insulin secretion, induction of insulin resistance). Future studies are necessary to elucidate the exact mechanisms and brain areas involved in mediating amylin's anorectic effect.

The third finding in this study was that amylin was also effective in eliciting an anorectic response in nondeprived young adult rats (age: 9 weeks). This is not in accordance with our earlier findings (20) where we reported that the anorectic effect of low doses of amylin was absent in nondeprived old rats (age: 15–18 months). However, recent studies have confirmed the anorectic effect of low doses of amylin in nondeprived young rats fed the medium-fat diet in an experimental setup in which feeding cost is relatively high (21a). The contradictory results may be explained by the use of young rats in the present but old rats in the former study.

Future studies will be necessary to investigate the physiological relevance of the observed anorectic effect of exogenously administered amylin. However, the present evidence that low doses of amylin similar to anorectic doses of cholecystokinin, a physiological satiety hormone producing meal ending satiety (15, 23), are effective in reducing food intake (20), that amylin seems specifically to affect food but not water intake (20), and the lack of evidence that amylin induces a conditioned taste aversion (7), favors the idea of amylin having a physiologically important role in the regulation of feeding behavior.

In summary, we have shown in the present study that low doses of amylin (1 μg/kg) injected IP elicit an anorectic effect at different times of the day which is not abolished by subdiaphragmatic vagotomy. Rather, vagotomy appeared somewhat to enhance amylin's anorectic effect under certain conditions. Amylin's anorectic effect was also observed without prior food deprivation in young adult rats.

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