The role of amylin in the control of energy homeostasis

Thomas A. Lutz

Institute of Veterinary Physiology, University of Zurich, Zurich, Switzerland; and Zurich Center for Integrative Human Physiology, University of Zurich

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Lutz TA. The role of amylin in the control of energy homeostasis. Am J Physiol Regul Integr Comp Physiol 298: R1475–R1484, 2010. First published February 10, 2010; doi:10.1152/ajpregu.00703.2009.—Amylin is an important player in the control of nutrient fluxes. Amylin reduces eating via a meal size effect by promoting meal-ending satiation. This effect seems to depend on a direct action in the area postrema (AP), which is an area rich in amylin receptors. Subsequent to the activation of AP neurons, the neural signal is conveyed to the forebrain via relays involving the nucleus of the solitary tract (NTS) and the lateral parabrachial nucleus (IPBN) to the lateral hypothalamic area (LHA) and other hypothalamic nuclei. While the NTS and IPBN seem to be necessary for amylin’s eating inhibitory effect, the role of the LHA has not yet been fully investigated. Amylin may also act as an adiposity signal. Plasma levels of amylin are higher in obese individuals, and chronic infusion of amylin into the brain reduces body weight gain and adiposity; chronic infusion of an amylin receptor antagonist into the brain increases body adiposity. Amylin increases energy expenditure in rats; this effect occurs under various experimental conditions after peripheral and central administration. Together, these animal data, but also clinical data in humans, indicate that amylin is a promising candidate for the treatment of obesity; effects are most pronounced when amylin is combined with leptin. Finally, recent findings indicate that amylin acts as a neurotrophic factor in specific brain stem areas. Whether this effect may be relevant under physiological conditions requires further studies.

energy expenditure; hindbrain; area postrema; trophic effect; leptin

In respect to its effect on eating, the general understanding is that peripheral amylin acts directly in the brain, where it activates distinct brain areas. The techniques used to delineate these pathways and major findings of these experiments will be briefly summarized. Second, amylin is an interesting peptide in the control of eating because of its potential role as an adiposity signal in addition to the well-described satiating action (30, 79). Evidence for the less recognized amylin action as a potential adiposity signal will be reported. This includes recent studies that indicate that amylin may also play a role in the control of energy expenditure.

The multiple signals in the control of eating interact (e.g., CCK and amylin; 10,38), and it is a generally acknowledged concept that the various controls of eating can be classified as adiposity, or tonic, signals that enhance the effect of satiation, or episodic/phasic, signals (6, 54, 77, 78). Whether this concept also holds up in the case of amylin in respect to its supposed dual role as satiating and adiposity signal is at present unknown. In other words, it is not known whether tonic levels of amylin affect the satiating effect of phasic, meal-associated changes in amylin levels. Of note, adult amylin-deficient mice1

1 Some of the studies reviewed here were performed with amylin-deficient mice and their wild-type controls. Amylin-deficient mice show a discreet phenotype of unaltered food intake, combined with a slightly higher rate of body weight gain compared to wild-type controls (17, 30, 38). The relevance of these findings will be discussed. Interestingly, endogenous amylin may have a facilitating role for other controls of eating. Amylin seems to mediate, in part, the central anorectic effect of CCK because CCK’s anorectic effect was...
eat less after acute peripheral amylin. In a recent experiment, we showed that an acute injection of amylin (50 μg/kg ip) significantly reduced eating in ad libitum-fed, amylin-deficient, and wild-type control mice to a similar extent (4-h food intake: wild-type NaCl 1.63 ± 0.13 g vs. amylin 1.25 ± 0.09 (76% of controls); amylin-deficient 1.45 ± 0.10 vs. 0.95 ± 0.23 (66% of controls)). Hence, at least the acute eating inhibitory effect of amylin does not require an underlying tone of endogenous amylin. Whether high tonic amylin levels increase the episodic effect of amylin on eating has not been tested.

Further, I will discuss recent data that suggest a role for amylin in brain development, specifically, in the development of hindbrain projections that are considered important to mediate amylin’s action on eating. Finally, I will address recent animal and early-stage human data in respect to amylin’s therapeutic potential in antiobesity therapy; in particular, its interaction with leptin to lower food intake, body weight, and adiposity is of high interest.

**Amylin Acts as a Satiation Signal**

In the complex system that controls eating, amylin functions as a potent satiation signal and is believed to be a physiological control of meal size (30, 31, 81). Certain criteria need to be fulfilled so that an endocrine factor is considered to be of physiological relevance (22); this requirement seems to be met by amylin for its satiation action. Eating (5-g test meal given to overnight fasted rats) leads to an immediate rise in endogenous plasma amylin levels from about 3–5 pmol/l to 15–20 pmol/l. Exogenous amylin has an acute onset of action and decreases eating in rats within a few minutes (31). Amylin’s effect relies on an amylin-induced reduction in meal size; this reduction occurs without signs of a conditioned taste aversion or without an increase in kaolin consumption (31, 34). Administration of the amylin receptor antagonist AC187 stimulates eating by increasing meal size, presumably by a blockade of endogenous amylin action (40, 50). The satiating effect of peripheral amylin seems to be mediated by direct action on area postrema (AP) neurons; neither subdiaphragmatic vagotomy nor capsac- icin-induced lesions of peripheral neural afferents that project to the brain reduced amylin’s effect, but this effect was abolished in rats with specific AP lesions. Further, local injection of amylin into the AP inhibited eating by reducing meal size, and AP injections of the amylin receptor antagonist AC187 had the opposite effect. The in vivo behavioral data are consistent with electrophysiological and immunohistochemical studies that confirmed a direct influence of amylin on the AP (30, 32, 40, 51, 52).

Amylin binds strongly to the AP (63), and all identified components of the specific amylin receptor complex, i.e., the calcitonin core receptor (CT-R), together with several receptor-activity modifying proteins (RAMPs) that confer amylin affinity and selectivity, are expressed in the AP. The RAMPs regulate the transport of the core receptors to the cell surface and their glycosylation state which determines ligand specificity (21, 36, 42, 71). The amylin receptor arises from the interaction of RAMP 1 or RAMP 3 with the CT-R, and both RAMP1 and RAMP3 mRNA have been discovered in the mouse AP (71). Further, amylin-induced c-Fos mRNA and RAMP3 mRNA expression colocalize in the rat AP (7), and amylin-sensitive AP neurons carry the CT-R (8). One critical experiment still needs to be done, i.e., to test whether the CT-R and pertinent RAMPs colocalize on the same, amylin-activated AP neurons.

**Central Processing of the Amylin Signal and Neural Pathways Originating in the AP**

Amylin primarily targets AP neurons. Cyclic guanosine monophosphate (cGMP) may be the second messenger mediating amylin’s effect in AP neurons because peripheral amylin markedly increased cGMP formation in the AP about 30 min after administration (51). Further, cGMP formation may be functionally relevant for amylin’s eating inhibitory effect because local AP injection of a membrane-permeable agonon of cGMP decreased eating by a meal size effect, similar to amylin (40).

AP activation is synaptically transmitted to the forebrain via the nucleus of the solitary tract (NTS) and the lateral parabrachial nucleus (IPBN) (52). Lesions of the AP, the NTS, or the IPBN blocked the eating-inhibitory effect of peripheral amylin; these lesions also blocked the amylin-induced c-Fos expression in areas rostral to the site of lesion, specifically, in the NTS, IPBN, and central nucleus of the amygdala (CeA) in AP-lesioned rats and in the CeA in IPBN-lesioned rats. Hence, evidence for the functional role of these pathways was defined by independent in vivo and in situ tests, including site-specific brain lesions, c-Fos immunocytochemistry as a marker of neuronal activation and retrograde and anterograde neuronal tracing studies (9, 47, 52, 59). According to our recent neuronal tracing studies, the IPBN, in particular, appears to act as an important relay station between the hindbrain and the lateral hypothalamic area (LHA), where amylin reduces the fasting-induced c-Fos expression (47, 52). The tracing studies also revealed ascending projections from the IPBN to other hypothalamic nuclei, such as the ventromedial hypothalamic nucleus (47); see also Ref. 39). So far, the role of these hypothalamic projection areas in respect to amylin’s inhibitory effect on eating has been investigated only incompletely. The same holds true for the motor circuitry that eventually connects the incoming amylin signal to motor controls of ingestion.

Interestingly, the central pathway of other gastrointestinal peptides that also inhibit eating seems to overlap widely with that of amylin, at least when characterized with the frequently used technique of c-Fos protein expression as a marker of neuronal activation. This is true for CCK, glucagon-like peptide 1, or peptide YY 3–36 (2, 30, 52, 55, 59). It is so far unclear how a specific behavioral response may be initiated despite similar central activation patterns.

Of note, and despite its common use, c-Fos immunocytochemistry bears some inherent weaknesses. c-Fos expression is typically studied after single, acute administration of the hormones. The c-Fos activation pattern only mirrors a snapshot image at one specific time point; further, because of the time required for c-Fos expression, there is usually a long delay
between the time when a stimulus-induced behavior occurs (typically within minutes for satiation signals) and when CCK protein can be detected (typically done between 60 and 120 min after the stimulus). A further shortcoming of this technique is that it only depicts neuronal activation but not neuronal inhibition. Hence, overall, the functional implications of c-Fos expression for hormonal action on eating behavior are unknown, and there may, in fact, be none in respect to satiating signals.

More recently used techniques to study brain activation patterns may circumvent some of the inherent problems of c-Fos immunocytochemistry. For example, the phosphorylated form of ERK 1/2 (pERK) can be used as a marker for neuronal activation. It has not only been shown that CCK induced ERK phosphorylation, but also that pERK formation appears to be necessary for the satiating effect of CCK (67). Recent pilot studies indicated that amylin increases pERK formation in a time- and dose-dependent manner (C. Potes, unpublished observation). Interestingly, the peak of pERK was observed about 15 min after peripheral administration of an anorectic dose of (5 μg/kg) of amylin, hence, at a time when amylin’s satiating effect is fully developed (31). Whether pERK formation is required for amylin action and whether amylin-induced cGMP formation (40, 51), and ERK phosphorylation are linked has not yet been studied.

Another alternative to c-Fos immunocytochemistry may be extensive in vivo imaging, such as functional magnetic resonance (fMRI). While the spatial resolution of the fMRI signal cannot compete with c-Fos or pERK immunocytochemistry, fMRI allows studying specific brain pathways repeatedly in the same animal, including the temporal component of signal transmission.

Amylin as an Adiposity Signal

Characteristics of amylin. It was already briefly mentioned that amylin shares characteristics of satiation signals, like CCK, but also of adiposity signals, like leptin or insulin (25, 62, 77, 78). Several lines of evidence provide plausible arguments for such a role of amylin as a potential adiposity signal.

First, the basal plasma levels of amylin are higher in obese vs. lean individuals, but the comparisons were interindividual comparisons, and amylin levels had not been measured in individual animals throughout the development of obesity (28, 46). Basal and glucose-stimulated plasma amylin levels are also elevated in obese humans (20, 24), and our own recent data indicate that this is also true in cats (35); lean and obese cats in that study were of similar age and body weight (4.6 ± 0.8 kg vs. 4.7 ± 0.8), but adiposity differed significantly (29 ± 1 vs. 37 ± 2% body fat per body weight); lean cats had significantly lower baseline amylin levels than obese cats (41 ± 9 vs. 56 ± 4 pmol/l).²

These findings support an association between body adiposity and plasma amylin, but several very important issues remain to be clarified. To mention just a few, it will be interesting to test whether changes in body adiposity result directly in changing amylin levels, whether these follow the same temporal pattern, and how short-term meal-related fluctuations of amylin may interact and overlap with an adiposity-driven pattern of plasma amylin levels.

Second, a number of studies showed that chronic peripheral (34, 56) or central (60) amylin infusion decreases body weight gain, specifically, by reducing fat mass. These effects may also be mediated by an action of amylin in the AP (33).

Third, chronic third ventricular administration of the amylin antagonist AC187 increased body adiposity without altering body weight (61). And finally, the amylin knockout mouse is heavier than wild-type controls (30); of note, crucial experiments, such as to test whether amylin replacement reverses this phenotype, have yet to be performed.

On the basis of these observations and with the reservation that some of the open questions will be answered in a way consistent with this idea, it appears plausible to consider amylin a potential adiposity signal. This idea was tested further in recent experiments (75). Rats were infused with amylin centrally into the third cerebral ventricle, and body weight was manipulated before amylin administration. In the first test, body weight manipulation consisted in a two-day total food deprivation. In this case of an acute decrease in body weight by short-term fasting, chronic central amylin infusion reduced food intake and body weight gain in a way similar to that in ad libitum-fed controls, i.e. irrespective of prior manipulation (75). This is, in principle, in line with a study performed in female rats receiving chronic peripheral amylin infusion; this study showed that both previously food-restricted rats (75% of baseline food intake for 10 days) and ad libitum-fed controls ate less and gained less body weight when receiving amylin (57). In our second test (75), body weight manipulation consisted in voluntary overfeeding by offering rats access to a highly palatable, energy-rich diet. We found that in the case of this marked increase in body adiposity by voluntary overfeeding, chronic central amylin infusion again reduced food intake and body weight gain, irrespective of prior manipulation (75). In fact, in both tests, amylin-infused animals appeared to reach a body weight that was similar to that of amylin-infused animals that were not manipulated before amylin administration (75). In other words, the central amylin level appeared to be an important determinant for the body weight to be reached. These results are, in principle, comparable to what has been reported for leptin or insulin (15, 78), and the data are compatible with the idea that amylin, like leptin or insulin, may encode the regulated level of body weight and hence may contribute to the relative constancy of body weight throughout adult life.

Resistance to adiposity signals. It is a well-known phenomenon that most cases of obesity in humans and in animal models of obesity, with the exception of leptin-deficient ob/ob mice, are typically associated with the development of resistance to the central effects of leptin and insulin. Leptin and insulin resistance is reflected by a reduced eating-inhibitory response to exogenous leptin and insulin administration, and by a shift in the respective dose-response curves. The suggested causes of this resistance phenomenon are numerous and seem to involve changes at the level of the blood-brain barrier because transport of leptin and insulin through the blood-brain barrier is reduced in obesity (3–5). Further, the cellular responses of hypothalamic neurons, which are one central target for leptin and insulin, are altered in obesity (43).
Reactive leptin, the obese Zucker rat, and the melanocortin-4 receptor knockout mouse (19, 23, 41). Antagonism to endogenous amylin with peripheral AC187 also increased eating in Zucker rats (23). Further, at least using high doses, recent reports in humans indicated that the acute administration of the amylin analogue pramlintide decreased the size of test meals by about 20% in nondiabetic obese individuals (14, 26). Hence, there is clear evidence that amylin is, at least, partly effective in obesity. However, no systematic studies have been performed so far to test whether the development of obesity or concomitant phenomena are paralleled by a reduction in amylin sensitivity; there is no evidence that hyperleptinemia or leptin resistance leads to amylin resistance.

Assuming that future studies suggest that amylin sensitivity may be reduced in obesity, it will be important to find out the potential mechanisms. It is well accepted that amylin is also transported across the blood-brain barrier (3), but it is unclear whether this transport, similar to leptin or insulin, is altered in obesity. Such transport may actually not be required for amylin action because the primary site of peripheral amylin action is supposed to be in the AP (32, 33). The AP is devoid of a blood-brain barrier, so that amylin has easy access to its receptors. Hence, other mechanisms would be more likely to explain any potential obesity-related amylin resistance. This may involve resistance at the cellular level such as a reduced expression of the CT-R or one of the critical RAMPs, disturbed CT-R/RAMP interactions, or postreceptor defects that lower the sensitivity of the amylin-signaling system.

**Dual Role of Amylin as Satiation and Adiposity Signal**

The two postulated roles of amylin as satiation and adiposity signals have been discussed (see Amylin Acts as a Satiation Signal and Amylin as an Adiposity Signal). This distinguishes amylin from the “classical” satiation hormone CCK. Continuous infusion of rats with CCK does not result in a sustained reduction in food intake and body weight (16); further, timed CCK infusions before spontaneous meals that reduce meal size are soon paralleled by a compensatory increase in meal frequency (72). Hence, an initial decrease in eating and body weight was rapidly counteracted by an increase in meal number that resulted in no net change of total food intake. In contrast, chronic amylin infusions reduce average meal size without this compensatory increase (1, 33). It is unknown whether the roles of amylin as satiating and as adiposity signals are processed differently by the central nervous system. One could imagine processing of the signals in different brain sites or by different neuronal populations in a given brain site. Similar questions may also be asked with respect to other controls of eating, like insulin or ghrelin (66, 76).

At least with respect to the primary site of action, it is known that the acute satiating effect and the chronic effect of peripheral amylin to decrease eating and body weight seem to require an intact AP (32, 33). We also know that the acute effect of amylin on eating is mediated by catecholaminergic neurons in the hindbrain (48). Whether the same neurons are necessary for the more long-term, body weight-lowering effect of amylin has not been tested.

**Amylin Increases Energy Expenditure**

Over recent years, several studies investigated the effect of enhanced amylin receptor signaling on energy expenditure. Body weight and body fat loss in fasted rats treated with repeated peripheral injections of the amylin receptor agonist sCT were more pronounced than in saline-treated controls, indicating that sCT most likely increased energy expenditure (29). Further, body fat loss in rats centrally infused with amylin was more pronounced than in pair-fed controls, again indicating a possible effect on energy expenditure, in addition to a reduction in food intake.

These findings are corroborated by independent and consistent reports that indicate that both acute and chronic amylin administration seems to influence energy balance by increasing energy expenditure as assessed by indirect calorimetry (27, 34, 44, 56). Chronic peripheral amylin administration increased total energy expenditure (34, 56). This effect may be secondary to the reduction in adiposity by amylin and hence, the relative increase in metabolically more active lean body mass (56). In a recent study, we showed that rats infused chronically via osmotic minipumps with peripheral amylin (6 μg·kg⁻¹·h⁻¹) had a total energy expenditure that was comparable to control animals. Despite lower average food intake and lower body weight at the end of the experiment, the average dark-phase energy expenditure over the 1-wk infusion period was 67.2 ± 0.6 kcal/kg in amylin-treated rats vs. 66.5 ± 0.5 kcal/kg in saline controls (n = 8). Importantly, energy expenditure in control yoke-fed to amylin-treated rats was significantly lower (62.3 ± 0.5 kcal/kg). In other words, amylin prevented the expected decrease in energy expenditure.

The results in regard to the effects of acute amylin administration on energy expenditure are less clear. For example, acute peripheral injection of an anorectic dose of amylin failed to increase energy expenditure in rats, while the long-acting amylin agonist sCT increased it (74). We presume that the lack of effect of peripheral amylin may be due to its short half-life in the peripheral circulation; after acute central administration when amylin presumably has a longer half-life at the site of action, low doses of both sCT and amylin increased energy expenditure by about 25%. On the basis of our findings and reports that central amylin specifically lowers body adiposity (60), we presume that amylin increases lipid metabolism, as indicated by a lower respiratory quotient.

Data about the central neural targets that may mediate the effect of exogenous amylin on energy expenditure are scarce. It has not been tested whether the AP, which seems to mediate most of amylin’s known effects, also plays a role in this regard. Further, the exact mechanisms that may underlie amylin’s effect on energy expenditure are also unknown. Most studies do not report a major effect of amylin on physical activity; hence, it is unlikely that this factor plays an important role (74). Further, an effect of amylin to raise body temperature was not seen consistently through all studies (44; see also Ref. 74 for effect of sCT). In some but not all studies, the effect of amylin on the expression of uncoupling protein was tested, but no major effect was reported (56). Of note, maintained body temperature at a given level of energy expenditure in animals...
that weigh less may indicate that amylin affected heat dissipation. However, this has not been tested.

Finally, it is important to mention that the physiological relevance of amylin’s effects on energy expenditure is still unsettled. It cannot be excluded that the effects summarized above only occur under pharmacological conditions because most of the criteria that are considered necessary requirements for physiological endocrine controls (e.g., 22) have not yet been tested in this respect. In other words, critical experiments investigating the role of endogenous amylin in the control of energy expenditure still need to be done; this may include experiments based on the use of amylin antagonists or amylin-deficient mice.

Nonetheless, we believe that the data reported above are consistent with the idea that amylin affects energy balance not only via an effect on food intake but also by increasing energy expenditure. At least according to some studies, this effect has to be seen in light of amylin’s body weight-lowering effect. Hence, we consider the effect of amylin to prevent the compensatory decrease in energy expenditure that is typically seen in weight-reduced animals or fasted humans (73) as indicative of a physiologically relevant effect on energy balance.

Trophic Effect of Amylin for the Normal Neural Development of the Brain Stem

Recent studies at our institute indicated that amylin may exert trophic effects for the normal development of brain stem neuronal pathways, specifically, for projections from the AP to the NTS. This phenomenon may be comparable to the effect of leptin in the hypothalamus that has been extensively investigated by Bouret and his colleagues (11–13). They showed that leptin seems to be required for the normal neuronal development of the neonatal mouse brain, specifically, in the hypothalamus. The implicated neural pathways are critical for the control of energy balance.

Bouret and colleagues (11, 12) specifically showed that genetically leptin-deficient Lep ob/ob mice and leptin-resistant diet-induced obese rats have deficient projections from the hypothalamic arcuate nucleus (ARC) to the paraventricular nucleus (PVN). Interestingly, normal mice that are not leptin deficient have an early postnatal surge of leptin secretion. This surge is obviously not present in Lep ob/ob mice, but this surge may be required for the normal development of the brain because peripheral leptin replacement in Lep ob/ob mice within the first few postnatal days appeared to restore the normal anatomical pattern of these ARC to PVN projections.

Our recent experiments were based on reports that amylin exerts trophic effects in a variety of tissues and organs. It had, for example, been shown that amylin influences the development of the kidneys, of bone, and of the pancreas (80). Using a similar methodology as Bouret and colleagues (11–13) with the DiI neuronal tracing technique, we tested whether amylin may also be an important trophic factor for the normal development of the mouse brain. Because of the primary action of amylin on AP neurons, we were specifically interested in the potential effect on hindbrain projections, namely, from the AP to the NTS in the early postnatal period.

We found evidence that amylin may be necessary for the development of AP-NTS projections in the mouse because genetically amylin-deficient mice had a markedly reduced density of AP-NTS projections compared with controls when tested on postnatal day 10 (53). Whether other brain areas are affected has not yet been tested in detail; however, first pilot studies indicated that amylin-deficient mice may also have a lower density of ARC to PVN projections, similar to Lep ob/ob mice.

Clearly, further experiments are warranted; for example, it will be important to find out whether amylin replacement therapy, similar to leptin (11), restores the normal pattern of AP to NTS projections and whether the effect of amylin on the development of these projections occurs prenatally or postnatally. It is, in principle, plausible that the effect of amylin occurs prenatally because maturation of feeding circuits in the hindbrain appears to precede that in the hypothalamus (64). Further, despite the obvious neuroanatomical defect in the brain of early postnatal amylin-deficient mice, it is currently unknown whether these projections are also deficient in adult amylin-deficient mice and whether they play a specific functional role in amylin’s inhibitory effect on eating or in other AP-mediated effects of amylin.

Interestingly, adult amylin-deficient mice do show an anorectic response to exogenous amylin administration (see Characteristics of amylin). Further, the amylin-induced neuronal activation in the AP and in the NTS, as gauged by c-Fos expression, is at least as prominent in amylin-deficient as in wild-type control mice (number of c-Fos-positive cells in the AP 2 h after amylin [20 μg/kg ip]: wild-type saline 3 ± 1 vs. amylin 42 ± 11 (P < 0.01); amylin-deficient 3 ± 1 vs. 68 ± 7 (P < 0.01)). This could mean that there is some structural or functional compensation for the defective AP to NTS connections that we observed in early postnatal amylin-deficient mice. Further, it is possible that the lack of endogenous amylin in amylin-deficient mice leads to a compensatory upregulation of amylin receptors in the AP that may increase the overall sensitivity of the system and hence may overcome the (relative) paucity of AP to NTS connections in these animals; both alternatives are interesting topics to be studied in the future.

Finally, we cannot exclude that these specific neural connections from the AP to the NTS are, in fact, not necessary for amylin’s acute anorectic effect or amylin’s effect to increase c-Fos expression in adulthood. Because our recent results indicate that catecholaminergic projections are necessary for the full eating inhibitory effect of amylin (48), it will be interesting to phenotype the AP to NTS projections that are developed to a lesser degree in amylin-deficient mice.

As mentioned before, the trophic effect of amylin in the hindbrain is also interesting because it shows parallels with comparable effects of leptin in the hypothalamus; in both cases, extensive experiments in other brain areas have not been performed yet. Both hormones are examples of integrative actions of signals that seem to influence both the brain structure (e.g., AP to NTS and ARC to PVN connections, respectively) and function. Whether leptin and amylin interact in the structural aspect of their action and whether the (mal)function of these systems is linked to metabolic diseases later in life remain to be studied (see e.g., Ref. 65).

Interactions of Amylin with Leptin: Animal Studies

Behavioral and metabolic effects—interaction of exogenous amylin and leptin. Because of the complexity of the system controlling energy balance and because of the multitude of
signals involved in this system, research on the potential interactions among these signals clearly is warranted. The interactions between amylin and leptin have been a special focus of research by several groups; results are encouraging in respect to the usefulness of this combination of signals in antiobesity therapy. Of note, we believe that this interaction is functional, i.e., not based on a direct effect of amylin at leptin receptors or vice versa, and that the neuroanatomical basis for this interaction, as well as intracellular signaling mechanisms, still need to be defined in detail. However, some light has been shed on the potential mechanisms that will be discussed here.

Our own recent studies showed that upon acute administration, central leptin increased the acute eating-inhibitory effect of peripheral amylin in rats (45). Several more chronic studies yielded results that are, in principle, consistent with our acute experiment. One of these studies (58) describes the effects on eating and body weight of combined two-week peripheral infusions of amylin and leptin in rats. Rats with diet-induced obesity were included; in these rats, exogenous leptin alone had no effect on food intake or on the development of body weight (of note, the same dose of leptin was effective in lean animals). Amylin alone reduced eating significantly in obese rats; this led to a decrease in body weight by about 5%. When the same dose of leptin was combined with amylin, both food intake and body weight were decreased more than by amylin alone; the body weight decrease was about double. Importantly, the body weight loss in rats that were pair-fed to the amylin-treated rats and that only received leptin was not more pronounced than in rats that were infused with amylin alone. In other words, additional exogenous amylin was necessary to increase the potency of leptin and to increase the relative body weight loss in obese rats. Similar effects were observed with respect to body adiposity because body fat was lower after coadministration of leptin and amylin than if rats received only amylin or if rats received leptin and were pair-fed to amylin-treated rats. Finally, dark-phase energy expenditure was highest in the rats that received both amylin and leptin. Overall, amylin infusion was necessary to enhance the leptin sensitivity of obese rats to these catabolic effects of leptin (58).

The same research group performed additional experiments with several dose combinations of amylin and leptin. These studies confirmed that amylin and leptin have synergistic effects on eating, body weight, and body adiposity. The strongest effect on body weight was a weight loss of about 15% (68, 70). This is a stronger decrease than is typically achieved with other, nonsurgical antiobesity treatments. At least under the conditions of one of these studies (68), the combination of amylin and leptin did not seem to have a major effect on energy expenditure because the body weight loss in rats that were pair-fed to the amylin- and leptin-treated group was similar. Nonetheless, the decrease in energy expenditure in pair-fed rats (compared to saline-treated ad libitum-fed controls) was not seen in the amylin- and leptin-treated group. Hence, similar to our recent study with central amylin infusion (75), the decrease in energy expenditure that might be expected due to lower body weight was prevented by amylin and leptin. Interestingly, the loss of body fat was more pronounced in rats that received amylin and leptin than in rats pair-fed to the treated rats; in other words, the synergistic effects of coadministration of amylin and leptin on fat pad size were more pronounced than the effects on body weight (58, 68). The effect to lower body fat is consistent with the observed effects of combined amylin and leptin treatment on nutrient metabolism; while the respiratory quotient was low during weight loss in both the amylin- and leptin-treated and the pair-fed groups, indicating preferential oxidation of fat, the respiratory quotient increased again in the pair-fed but not in the amylin- and leptin-treated rats when lower body weight was reached and when the body weight stabilized at this lower level. Gene expression profiles were also consistent with these metabolic effects in the amylin- and leptin-treated rats; the expression of genes for hepatic lipogenesis was reduced, and the expression of genes for lipid utilization was increased (68).

Mechanism(s) of Interaction

Recent studies have elaborated on the potential mechanisms of how amylin and leptin may interact; these studies indicate that amylin influences the central processing of the leptin signal (58, 70). Importantly, the studies indicate that amylin not only reverses leptin resistance in obese rats (58) but also increases leptin sensitivity in lean, leptin-responsive rats (70). These studies also indicate that the AP, which presumably is amylin’s primary target area, does not seem to be the major converging site for this interaction. When lean rats received single injections of leptin and amylin, leptin did not enhance the amylin-induced activation of AP neurons as gauged by c-Fos expression (70). Interestingly, rats that were pretreated with amylin for 1 wk showed increased pSTAT3 formation—the activated form of STAT3—in the AP after acute leptin (58). However, the effect was relatively weak, and a very large dose of leptin had been used in that study using diet-induced obese rats.

Rather than in the hindbrain, the amylin-leptin interaction appears to reside in the hypothalamus, possibly after polysynaptic input from the AP, which primarily senses the amylin signal (32, 33, 70). When lean rats received acute injections of a low dose of leptin and of amylin, amylin increased the effect of leptin to induce pSTAT3 formation in the ARC but not in other brain areas (70). Further, at least high doses of leptin resulted in increased pSTAT3 signaling in the ventromedial hypothalamus (VMH) in obese, amylin-pretreated rats (58); in other words, amylin restored the leptin-induced immunoreactivity of pSTAT3 in the VMH of obese rats to a level that is seen in leptin-treated lean rats. Hence, amylin overcame leptin resistance in obese rats at the cellular level.

These results are consistent with our unpublished pilot studies in which we showed that acute amylin treatment up-regulated leptin receptor expression about three-fold in the rat hypothalamus. Further, leptin binding, as determined by receptor autoradiography, in the rat brain was increased in the ARC by combination treatment with amylin and leptin, and it was increased by amylin alone in the VMH and the dorsomedial hypothalamus (DMN). Finally, these results are also consistent with reduced leptin receptor expression in the mediodiagonal hypothalamus in amylin-deficient mice (see below; 70). It is plausible that the effects of amylin on pSTAT3 expression and on leptin receptor expression are causally linked and are part of a common neuromechanism, but this has not been investigated yet.

The finding that amylin and leptin may, at least in part, interact via the VMH is also interesting in the context of our
previous studies that had shown that amylin’s eating-inhibitory effect in rats is reduced by administration of the histamine H1 receptor antagonists pyrilamine or chlorpheniramine into the ventromedial hypothalamic nucleus; further, the acute effects of both amylin and leptin on eating were blunted in H1 receptor-deficient mice (37, 39). Hence, the potential role of histamine and of H1 receptors in the functional interaction of amylin and leptin in the VMH clearly deserves to be studied.

Overall, there is convincing evidence that the functional interaction between amylin and leptin involves the hypothalamus, possibly the ARC or the VMH. Leptin-induced pSTAT3 immunoreactivity was also increased by amylin in the AP in obese rats (58), but the effect was weak. Finally, amylin increased leptin receptor expression in the DMN, but leptin-induced pSTAT3 formation in the DMN was unaltered in amylin-deficient mice (70). It is clear that more studies are necessary to define the sites and mechanisms of amylin and leptin interaction.

**Interaction of endogenous amylin and leptin.** As outlined, there is clear evidence to date of a pharmacological interaction between amylin and leptin. A less investigated field is whether this interaction is also of physiological relevance; in other words, it is important to know whether endogenous amylin and leptin also interact in respect to their effects on eating and body weight. A recent study showed that amylin-deficient mice have a reduction in the leptin-induced pSTAT3 formation in the ARC and VMH and in leptin receptor expression in the mediobasal hypothalamus. Further, the body weight-lowering effect of chronic leptin was less in these mice compared to wild-type controls; similarly, the effect of leptin to decrease body adiposity was less in amylin-deficient mice, in particular, in male animals (70). Finally, the amylin agonist sCT seems to be less effective at reducing eating in leptin-deficient ob/ob mice (19), indicating that endogenous leptin may also be required for a full action of amylin. Overall, these experiments indicate that the functional interaction between amylin and leptin may also be of physiological relevance. Further studies with amylin-deficient and leptin-deficient mice that receive specific hormone replacements at physiological doses will be required to fully investigate the relevance of this interaction under physiological conditions.

**Therapeutic Potential of Amylin in Antiobesity Therapy—Human Studies**

Clinical studies. The findings discussed on the interaction between amylin and leptin in rodents are an interesting example of promising animal work and its translation into potential applications in humans. Preclinical tests in humans showed that the coadministration of the amylin mimetic pramlintide and the leptin mimetic metreleptin in overweight and obese humans resulted in a markedly reduced body weight (49, 58). This reduction was clinically relevant. The individuals were first treated with pramlintide alone; 4-wk pramlintide treatment resulted in a body weight loss of about 5%. At this time, three treatment groups were formed. Treatment was either continued with pramlintide alone or switched to metreleptin alone for another 20 wk. These individuals continued to lose body weight, resulting in a total body weight loss of about 8%. A third group of individuals received the combination of pramlintide and metreleptin; this treatment led to a weight loss of more than 12%, and most importantly, body weight did not yet stabilize at the end of the observation period. In other words, lower body weight was not only maintained, but body weight continued to decrease even after 24 wk of treatment.

Despite these promising results, there is no indication as yet that lower body weight in previously overweight individuals would be maintained on the cessation of amylin and leptin therapy. Recent experiments in diet-induced obese rats, in fact, indicated that without continued amylin and leptin treatment, body weight may increase again; the body weight loss was only maintained in rats that received continued treatment (69).
It should not be overlooked that despite the promising outcome of pharmacotherapy with amylin or its analogue pramlintide in the treatment of obesity, this effect has not yet been clearly linked to one or several of amylin’s potential physiological functions described above. In other words, while reduced eating (perhaps by an enhanced satiation effect) is involved (14, 26, 58), it is not yet clear whether the body weight-lowering effect of chronic pharmacotherapy with pramlintide in humans is also based on higher energy expenditure, enhanced adiposity signaling (which may be mediated by other brain areas than the satiating effect), or a structural trophic effect that may underlie the increase in leptin sensitivity. None of these issues has been tested so far.

Outlook. Both the animal and the human work suggest that the combination treatment with amylin and leptin (or their analogues) is a promising approach in the treatment of human obesity. In fact, the body weight loss induced by this combination approached that of bariatric surgery, which is still the most effective body weight-lowering treatment for obesity available to date. Hence, the combination of amylin and leptin may be the best nonsurgical approach available so far. Whether lower doses of amylin or leptin (or their mimetics, respectively), can be used to maintain lower body weight once marked body weight loss has been achieved, is not known yet. Further, potential long-term side effects of this combination treatment still need to be explored.

As discussed in Amylin Acts as a Satiation Signal, the amylin receptor consists of a heterodimer of the CT-R core receptor and RAMP1 or RAMP3 (36, 42). These RAMPs may be another interesting potential avenue to develop antiobesity therapy that involves modification of the amylin signaling system. In fact, RAMP-based therapy may potentially have only little side effects because of the relatively specific role of RAMPs. The only well-characterized role of RAMPs so far is that they are key components for receptors of the amylin family of peptides. Theoretically, drugs that enhance the formation of functional CT-R and RAMP complexes or that specifically enhance the effect of RAMP signaling may enhance the action of amylin without producing unspecific side effects.

Summary

Amylin is a physiological satiating signal that controls meal size. Experimental evidence clearly indicates that this action is triggered by amylin receptors in the AP; subsequent activation involves the NTS, IPBN, and other brain areas (see Figure 1 for our current working model). Chronic amylin treatment decreases eating and body weight gain in both experimental animals and humans. Amylin antagonists increase eating and body weight gain in rats. These and other data suggest that amylin may also function as an adiposity signal. Recently, we showed that amylin may influence the brain structure because amylin may be required for the early postnatal development of the hindbrain due to its positive neurotrophic effect. Finally, animal and human studies with amylin and leptin (or synthetic mimetics) suggest that this combination therapy may be an effective treatment for obesity.

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DISCLOSURES

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REFERENCES

AMYLIN AND ENERGY HOMEOSTASIS


