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ORIGINAL ARTICLE

Effect of amylin on prolactin release

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Abstract

OBJECTIVE: Amylin is a 37-amino acid peptide that is secreted from the pancreatic β cells. This peptide is cosecreted with insulin from the pancreas by glucose. Amylin plays a role in glucose homeostasis and in the regulation of lipid metabolism.
Amylin receptors were identified in central nervous system of rats. There is no available data on the effects of amylin on the pituitary hormones secretion.
The aim of this study was to evaluate the effect of amylin on prolactin release in vivo and in vitro experiments in male adult Wistar Kyoto rats.
METHODS: Amylin in a dose of 1μg/5μl was injected intraventricularly (i.c.v) during 5 min. using automatic pump. The control group received 5 μl cerebrospinal fluid. Amylin was injected intravenously (i.v) in a dose of 10 μg in 300 μl 0,9% NaCl. The control group received 300 μl 0,9% NaCl. In vitro experiment was performed in the pituitary cells culture conditions. Amylin was added to pituitary cell culture in a dose of 1, 10, 100 nM. Prolactin concentrations were determined using RIA methods.
RESULTS: Central or peripheral administration of amylin caused a significant decrease of serum PRL concentrations as compared with control after 120 min.
After 240 minute incubation of the pituitary cells culture with amylin in doses of 10 nM and 100 nM a significant inhibition of the release of PRL was found. However we found that the effect of amylin on the release of PRL depended on dose and time of incubation. A significant increase of PRL level was observed in cultured media in the presence of 1 nM of amylin after 60 min.
CONCLUSION: Our results indicate that amylin administrated centrally and peripherally as well in the cell culture inhibits PRL release.

Introduction

Amylin (islet amyloide polypeptide; IAPP) is a 37 aminoacide peptide that is secreted from the pancreatic β cells [1]. The data of Hanabusa at al [2] revealed that IAPP is cosecreted with insulin from the pancreas. The physiological role of amylin have not been exactly establish. Previous studies have demonstrated an effect of IAPP on glucose metabolism in several species of animals [3, 4, 5, 6, 7, 8, 9]. Authors of previous studies suggested that amylin is unlikely to be of physiological importance in peripheral glucose metabolism [5]. In all of theses studies the doses of amylin used were much higher above the physiological range. Arnello at al. [10] using a novel aortic cateterisation technique observed that chronic low dose amylin infusion reduces food intake, but has no influence on glucose metabolism. However the results of Wang at al [11] indicated that amylin is more potent and more effective than glucagon in raising plasma glucose concentration in fasted rats. Amylin infusion raised both glucose and insulin concentrations and these results may suggest that amylin can induce peripheral insulin resistance [12]. However some authors suggested [13] that hypoamylinemia rather then hyperinsulinemia per se can have directly caused the insulin resistance in the obese LA/N cp rats (insulin resistant Lister Albany rats).

Hettiarachchi [14] demonstrated that the specific amylin antagonist, amylin [8–37]; enhances whole body, liver, and muscle insulin sensitivity with a concomitant decrease of basal plasma insulin in both normal and insulin – resistant, hGH-infused rats. They found that amylin [8–37] infusion was associated with altered lipid distribution. Ye. J. M. et al. [15] observed that amylin stimulates lipolysis in vivo. These results may suggest that amylin plays a role in glucose homeostasis and in the regulation of lipid metabolism.

Moreover amylin inhibits food intake and gastric acid secretion [16]. Amylin receptors were identified in central nervous system in rats [17]. There is no available data on the effects of amylin on the pituitary hormones secretion.

The aim of this study was to evaluate the effect of amylin on prolactin release in vivo and in vitro experiments in male Wistar Kyoto rats.

Material and methods

Male Wistar-Kyoto rats (240–260 g) were maintained under controlled conditions (14L:10D, lights on at 06.00h, temperature at 23 ±1°C) with free access to food and water.

All experimental procedures were approved by the First Warsaw Ethic Committee for Experiments on Animals (the M. Nencki Institute of Experimental Biology, the Polish Academy of Science).

Intracerebroventricular (icv) administration of amylin

The animals were anesthetized ip with ketamine and implanted with a stainless-steel guide cannula, 23 gauge cannula was located in the third cerebroventricle (0.8 mm posterior and 7.0 mm ventral to the bregma at the midline) according to the atlas of Paxinos and Watson [18] The inside of the cannula was closed by a removable stainless-steel plug. The placement of the intracerebroventricular cannula was verified by an injection of methylene blue dye after decapitation. The brain was inspected for complete spread of the dye in the third ventricle.

After the surgery, the rats were transferred to individual cages with food and water freely available. During a 5-day period of recovery, rats were handled daily to minimize any stress associated with handling on the day of the experiment.

On the day of the experiment, 1 h before amylin administration, stainless-steel guide cannula were opened and their potency was controlled. Intracerebroventricular infusion of amylin was performed to freely moving rats. Amylin at a concentration of 1 μg in 5μl vehicle (artificial cerebrospinal fluid -CSF) or equal volume of the vehicle was slowly (1μl/min) infused into the thrid ventricle with an automatic pump (CMA/100; Sweden) through an inner cannula inserted into the guide cannula. After the end of the infusion the rats were transferred to their home cages with free access to food and water. At 60 and 120 min after the infusion of amylin or vehicle, animals were decapitated and trunk blood was collected in plastic tubes containing 1000 IU aprotinine (inhibitor of protease) per each ml of blood. The time-span from removal of the animals from their cages to decapitation was approximately 2 min.

Intravenous (iv) injection of amylin

Amylin in a dose of 10 μg in 300 μl of saline or 300μl of saline alone was injected into the tail vein. At 60 and 120 min after the injection of amylin or saline, animals were decapitated, and trunk blood was collected in plastic tubes containing 1000 IU of aprotinine / ml of blood

The blood samples were centrifuged (3000 rpm for 20 min at 4°C). Serum samples were frozen until hormonal analysis was performed.

Cultured of the pituitary cells at the presence of amylin

The method of pituitary cell culture was based on principles according to [19,20,21] and it was published in details previously by Baranowska et al.[22]

The pituitary cells (0.2 x 106 /ml) were incubated in 24-well culture plates for up to 48 hrs in a humidified atmosphere of 95% air and 5% CO2 at 37°C. Following, amylin in doses 1 nM, 10 nM or 100 nM were added and the medium was collected 60, 120 or 240 min thereafter. The collected medium was stored at – 20°C until assayed for PRL.

Hormone measurements

Serum concentrations of PRL were measured by RIA using reagents prepared by Dr. A.F. Parlow and provided by the NIDDK (Bethesda, MD). The detection limit for PRL assay was 0.5 ng/ml.

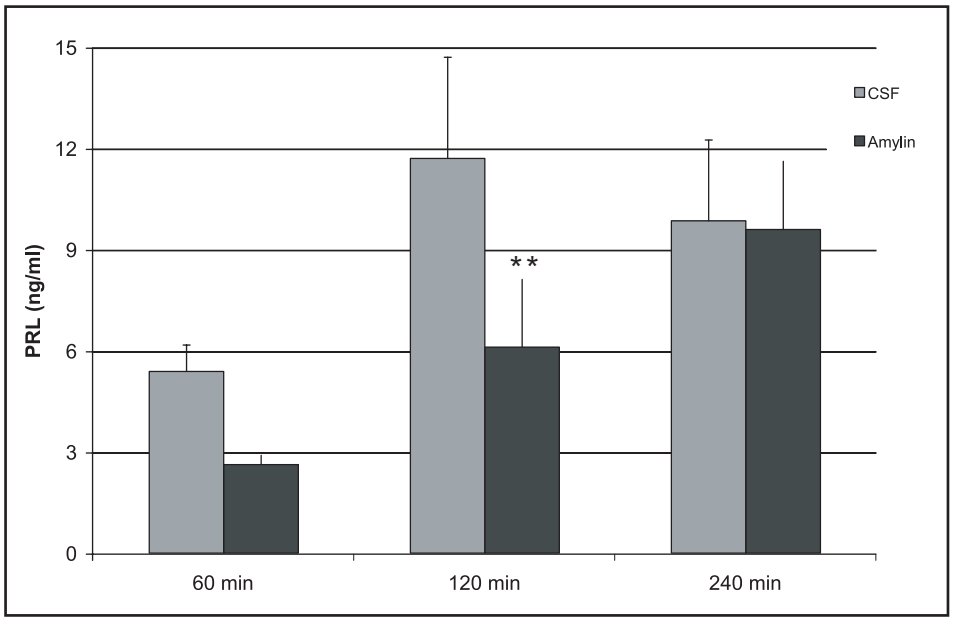

Figure 1. Effects of amylin on PRL after intracerebroventricular (icv) administration.
**p< 0,05

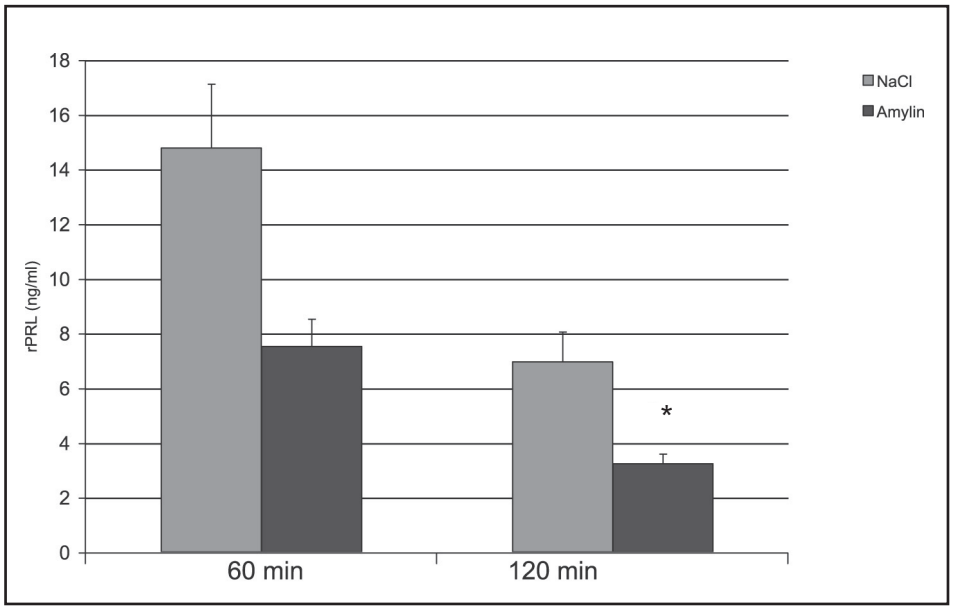

Figure 2. Effects of amylin on PRL after intravenous (iv) injection.
*p<0,01

In vivo experiment results

Effects of amylin on PRL after intracerebroventricular (icv) administration and intravenous (iv) injection are presented in figures 1, 2.

A significant decrease of PRL in the peripheral blood compared with control after 120 min ($p<0.05$) after the central administration (icv) of amylin was observed. Intravenous (i.v) injection amylin significantly decreased PRL level after 120 min. ($p<0.01$)

In vitro experiment results

Effects of amylin on PRL from cultured pituitary cells are presented in figure 3. The effect of amylin on the release of PRL was dependent on dose and time of incubation. A significantly increase of PRL level in cultured media at the presence of 1 nM of amylin after 60 min was observed ($p<0,01$ vs. control). However, after 240 min incubation of the pituitary cells culture in the presence of amylin in doses of 10 nM and 100 nM a significant inhibition of PRL release as compared to the control was found ($p<0,01$).

Discussion

It has been known that amylin displays 50% homology with calcitonin gene-related peptide (CGRP) and it is co localized with somatostatin in endocrine cells of the gastric fundus. Amylin participates in the regulation of gastric endocrine (somatostatin, histamine)

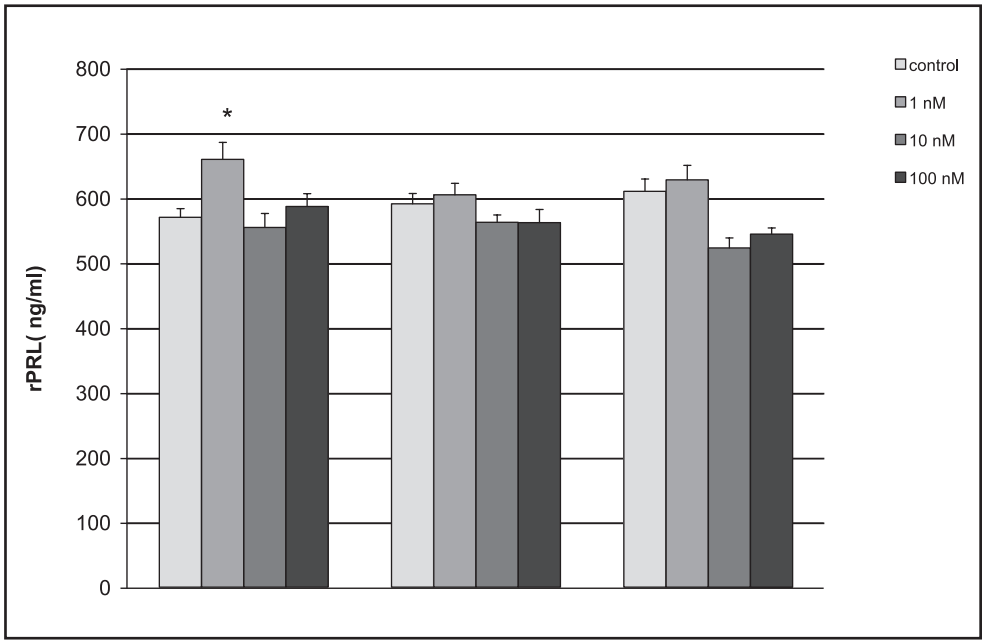

Figure 3. Effects of amylin on PRL in cultured pituitary cells.
*p<0,01

and exocrine (acid) secretion. Amylin enhances somatostatin secretion via autocrine mechanism and leads to inhibition of histamine and acid secretion [23].

Amylin antagonizes insulin action and causes in vivo insulin resistance, but amylin did not affect the level of plasma glucagon, epinephrine, norepinephrine and corticosterone and metabolism clearance rate of insulin [24]. Amylin modulates aminergic neurotransmiters in the hypothalamus and inhibits food intake, through inhibition of dopamine release without effecting norepinephrine or serotonin [25].

Moreover, amylin inhibits NPY, a potent feeding stimulating peptide, and leads to weight loss [26].

Nyholm et al. [27] demonstrated that amylin analog AC 137 caused a rise in circulating cortisol and GH release during hypoglycemia in patients with insulin – dependent diabetes mellitus.

In our experiments we observed that amylin administered centrally (icv) and peripherally (iv) produced a significant decrease in prolactin release. However, effects of amylin on PRL release from cultured pituitary cells were dependent on dose and time of incubation. We found inhibiting effects of amylin (in doses 10; 100 nM) on PRL release after 120 and 240 min. of incubation. Transitory stimulating effect of amylin in a dose of 1nM was observed only after 60 min. of incubation.

Paganii at el. [28] investigated the effect of amylin and salmon calcitonin (sCT) on β endorphin secretion induced GH and PRL secretion in male rats. They found that amylin inhibited β endorphin – that induced GH secretion. Where as sCT was able to inhibit β endorphin induced prolactin secretion. Amylin and sCT may act through various receptors and this finding may explain the differences in action on GH and PRL release.

Some factors may be involved in the mechanism of inhibiting effects of amylin on PRL release in our experiments in vivo and in vitro. It has been reported that amylin modulates neurotransmitters and neuropeptides activity. Effects of amylin on dopaminergic activity NPY (Neuropeptide Y) and VIP (vasoactive intestinal peptide) activity may be involved in the inhibition of PRL release. Fernandez at al. [29] indicated that IGF I and VIP induce lactotrophys proliferation and PRL release.

Conclusions

Direct and indirect inhibitory effects of amylin on PRL release were found.

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