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Review

Islet amyloid polypeptide in the islets of Langerhans: friend or foe?

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Abstract

Islet amyloid polypeptide (IAPP), or amylin, was originally discovered as the constituent peptide in amyloid occurring in human insulinomas and in pancreatic islets in human subjects with Type II (non-insulin-dependent) diabetes mellitus. Its normal expression in beta cells and its co-secretion with insulin in response to nutrient stimuli, suggest a metabolic function for the peptide. Specifically, IAPP has most frequently been shown to inhibit insulin secretion, implying that IAPP has a role in the regulation of islet hormone homeostasis. The physiological significance of IAPP in islets has been difficult to assess; very high IAPP concentrations are required to alter insulin secretion. Moreover, until recently, IAPP receptors have not been characterised at the molecular level, thus leaving the actual target cells for IAPP unidentified. Furthermore, in experimental diabetes in rodents, the ratio of IAPP expression to that of insulin invariably is increased. In view of the pleiotropic effects attributed to IAPP, such regulation could be both adverse and beneficial in diabetes. Metabolic characterisation of mice carrying a null mutation in the *IAPP* gene or which overexpress IAPP in beta cells have recently confirmed that IAPP is a physiological inhibitor of insulin secretion. Based on experiments in which IAPP-deficient mice develop a more severe form of alloxan-induced diabetes, we argue that the action of IAPP in the islets normally is beneficial for beta-cell function and survival; thus, the established up regulation of IAPP expression compared with that of insulin in experimental rodent diabetes could serve to protect islets under metabolically challenging circumstances. [Diabetologia (2000) 43: 687–695]

Keywords Alloxan-induced diabetes, beta-cell survival, calcitonin receptors, glucose tolerance, *IAPP* null mutant mice, insulin secretion, receptor-activity-modifying protein (RAMP), transgenic overexpression.

Introduction

Exactly one century ago, Opie [1] reported on the relation of interstitial pancreatitis to diabetes mellitus. Later, this morphological phenomenon was shown to represent a specific form of pancreatic islet amyloid, and to be a histopathological hallmark in human Type II (non-insulin-dependent) diabetes mellitus [2, 3]. Purification of the amyloid fibril protein in the 1980s from insulinomas and amyloid-rich pancreases from Type II diabetic patients [4–6] revealed a 37-amino-acid-long peptide with neuropeptide-like features, which has been designated islet amyloid polypeptide (IAPP), or amylin. In humans and in most other mammals studied to date, IAPP is predominantly expressed by the pancreatic beta cells in which it is stored together with insulin in dense core secretory granules [7–10]. The expression of IAPP can also occur in other locations such as the gut [11] and in the sensory nervous system [12]. Based on

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Abbreviations: CGRP, Calcitonin gene-related peptide; CRLR, calcitonin receptor-like receptor; CT, calcitonin; CTR, calcitonin receptor; h, human; IAPP, islet amyloid polypeptide; NOD, non-obese diabetic; RAMP, receptor-activity-modifying protein.

structural and functional similarities and on genomic analyses, IAPP is considered to be a member of the calcitonin family of peptides, which also includes calcitonin (CT), the calcitonin gene-related peptides (CGRPs) and adrenomedullin [13–16]. Although the physiological importance of the members of the CT family is not fully understood, treatment with IAPP or CGRP results in several similar biological effects [17]. One such effect is their ability to modulate insulin secretion. Among the CT family members, only IAPP and CGRP have been reported to occur in pancreatic islet cells; IAPP is expressed in beta cells and somatostatin cells whereas CGRP occurs predominantly in somatostatin cells and in sensory nerve fibres [12, 18]. It has therefore been conceived that these two peptides are involved in the regulation of insulin secretion. Here, most data available point to an insulinostatic action for IAPP.

Under diabetes-like experimental conditions in rodents, islet expression of IAPP is up regulated relative to insulin expression; in view of the effects attributed to IAPP, such regulation could be either adverse or beneficial in diabetes. The biological significance of the insulin-regulatory action of IAPP and the alterations in IAPP-expression in experimental diabetes are discussed in this review, with special reference to studies in transgenic mice lacking IAPP or overexpressing the peptide.

The effect of IAPP on insulin secretion

One of the most frequently investigated, and controversial, effects of IAPP is its ability to influence insulin secretion. Although conflicting data exist [19, 20], numerous studies have shown that IAPP treatment inhibits insulin secretion from perfused rat pancreases [21, 22], isolated pancreatic islets [23, 24] or isolated beta cells [25]. The physiological relevance of these inhibitory effects has, however, been questioned because of the high concentrations of IAPP required to affect insulin release, concentrations that exceed the normal picomolar concentrations found in the peripheral blood circulation. To affect the insulin response to glucose in humans IAPP concentrations 90-fold higher than normal postprandial peaks are required [26]. Yet, although the precise concentration of endogenous IAPP in the islet interstitium is not known, its amount in the human pancreas (600–1400 ng/g wet weight [27]) implies a much higher concentration of the peptide in the islets than in the peripheral circulation. Thus, high doses of exogenously given IAPP could be required to overcome a pre-existing tone of the endogenously released peptide.

Clarifying in this issue and in support of an insulinostatic action for IAPP are studies in which agents have been used that suppress the action or the expression of IAPP. Along these lines, it has been reported that treatment with human $CGRP_{8–37}$ (amino acids 8 to 37) or $IAPP_{8–37}$, both of which are used as IAPP antagonists [28–30], in themselves augment insulin secretion both in vitro and in vivo [30–33]; a similar approach, using IAPP-antiserum, potentiates arginine-stimulated or glucose-stimulated insulin secretion from isolated rat islets [33]. In HIT-T15 cells, a hamster insulinoma cell line, suppression of synthesis and release of IAPP with IAPP-antisense oligonucleotides are associated with increased insulin mRNA and content [34]. Taken together, these data point to an insulinostatic function for IAPP that seems to be mediated though autocrine/paracrine mechanisms in the islets.

Insulin secretion in IAPP transgenic mice

The generation and metabolic characterisation of *IAPP* null mutant mice or mice overexpressing human (h) IAPP in beta cells have recently been undertaken by us [35] and by others [36]; the insulin-secretion phenotype in both these transgenic animal models provides firm evidence of an insulin-counter regulatory action for IAPP. Male *IAPP* null mice subjected to oral or intravenous glucose tolerance tests (OGTT; IVGTT) have abnormally increased plasma insulin concentrations and a more rapid plasma glucose elimination compared with wild-type control males [35] (Fig. 1). The metabolic abnormalities in *IAPP* null males during OGTT or IVGTT seem specifically related to the lack of IAPP in beta cells because both plasma insulin and glucose perturbations are reversed after tissue-specific recovery of IAPP expression in beta cells [35]; this "phenotypic rescue" was achieved by introducing a rat-insulin promoter-driven h*IAPP* gene into the *IAPP* null strain. In contrast to males, *IAPP* null females have normal plasma insulin responses in response to glucose loading. Nevertheless, the female mutants have improved glucose tolerance compared with wild-type females after either oral or intravenous glucose loading. Thus, it is still to be resolved whether the metabolic phenotype in the female mutants shows a function for IAPP in peripheral glucose handling, a function which has previously been attributed to IAPP [37–39]. An alternative explanation is that the enhanced plasma glucose elimination in female mutants represents loss of IAPP expression (and function) in the gastrointestinal epithelium where IAPP is normally expressed [11]. The latter explanation is supported by increased glucose tolerance observed in female mutants not being affected by tissue-specific recovery of IAPP expression in beta cells [35]. More studies will be required to assess whether extrapancreatic mechanisms contribute to the improved glucose tolerance observed in the *IAPP* null strain.

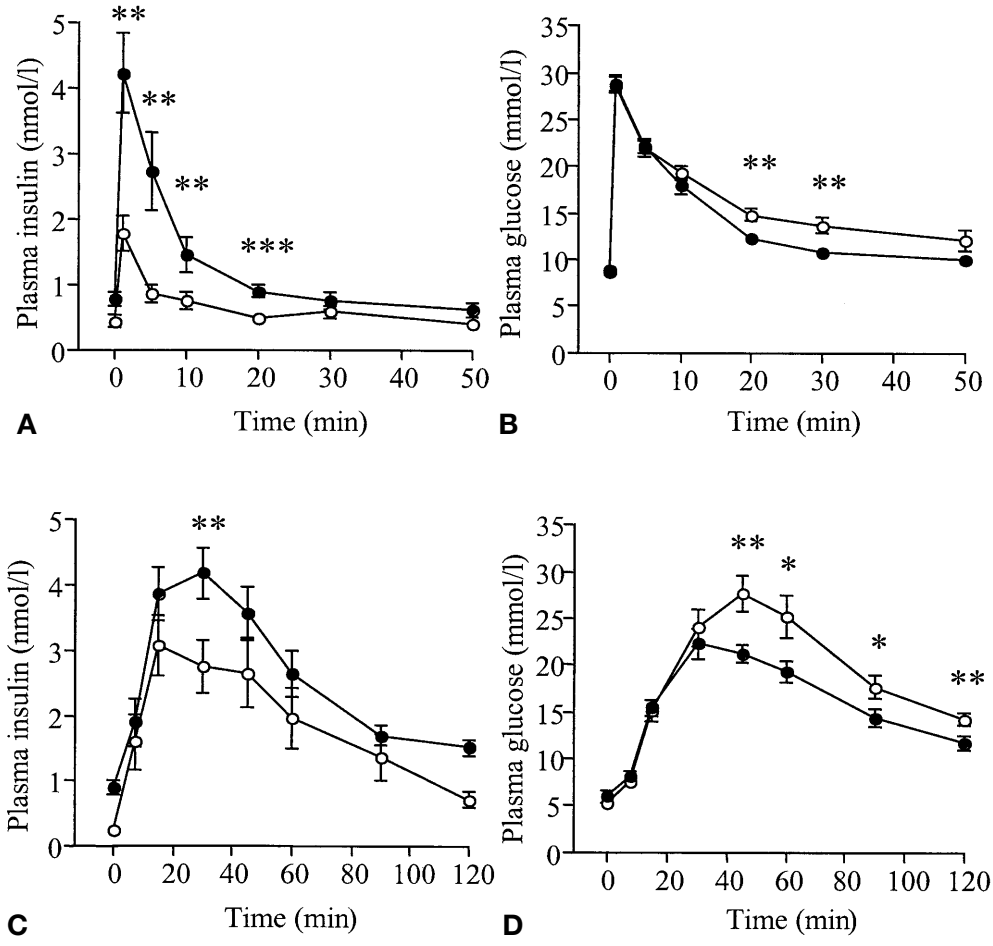

Fig. 1 A–D. Glucose challenge tests in *IAPP* null mutant males (black symbols) and wild-type control males (white symbols). The *IAPP* null mutants have higher plasma insulin concentrations in IVGTT or OGTT (**A**, **C**, respectively). Plasma glucose concentrations during IVGTT (**B**) and OGTT (**D**) are lower in the *IAPP* mutants. Values are means ± SEM, $n = 12$ in each group. ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$ compared with wild-type control males. Data from [35]

In accordance with the increased glucose-stimulated insulin responses and improved glucose tolerance detected in *IAPP* null males, hIAPP transgenic mice of both sexes show reduced plasma insulin responses and reduced glucose tolerance after glucose loading [36]. Thus, with the exception of the female null mutants, the two transgenic animal models uniformly provide evidence for a physiological function for IAPP in rodent glucose homeostasis; here, IAPP inhibits both insulin secretion and the rate of glucose disposal, the former probably being the cause of the latter effect. Notably, and in contrast to the male *IAPP* null phenotype which is presented in both OGTT and IVGTT, the hIAPP transgenic phenotype is seen only after oral glucose gavage [36]. The latter finding was argued to suggest that IAPP exerts some of its insulinostatic action through effects on the intestinal tract or the gut-islet axis or both. Further studies, for example using insulin-clamp techniques, are required to define the nature of altered glucose handling in both the *IAPP* null and hIAPP transgenic strains.

IAPP receptors

The assumed autocrine/paracrine inhibition of insulin secretion exerted by IAPP (see above) implies the existence of IAPP receptors on the surfaces of one or several cell types in the islets. Yet, until recently, the molecular structure of IAPP receptors has not been known. This significant lack of knowledge has hampered interpretation or definitive conclusions on the function of IAPP. The identification of specific IAPP receptors has therefore been a major challenge in the field of IAPP biology. Attempts to expression clone IAPP-receptor genes, using a selective ligand, have resulted in the isolation of cDNAs encoding the calcitonin receptor (CTR) [40]. Indeed, IAPP activates the porcine CTR when this receptor is expressed in some, but apparently not all, cellular host systems [41, 42]. Moreover, IAPP-binding sites partially overlap with those of CT and α-CGRP in the central nervous system [43].

The great similarities between CT and especially IAPP and the CGRPs [17] regarding their biological activities have indicated that the peptides might activate a group of similar, or even identical, receptors [44]; the availability of each ligand would thus be a determinant of which peptide is physiologically active in a given tissue. Members of a previously unknown group of receptor-activity-modifying proteins (RAMPs) were, however, recently found to interact with the orphan calcitonin receptor-like receptor (CRLR) and determine its ligand specificity [45]; whereas the combination of RAMP1/CRLR results in a CGRP receptor, RAMP2/CRLR is an adrenomedullin receptor. During 1999, two groups [46, 47] reported that heterodimers between the CTR and RAMP1 or RAMP3 preferentially bind IAPP; hence CTR/RAMP1 or CTR/RAMP3 could prove to be functional IAPP receptors. If this holds true, the identification of cells that coexpress the CTR and RAMP1 or RAMP3 will show the actual target tissues for IAPP. Although the existence of other IAPP receptors should not be ruled out at this point, the complex ligand-receptor interactions within the CT peptide family seem to explain previous difficulties in isolating a single IAPP-specific receptor protein.

IAPP and diabetes

Ever since the discovery of IAPP in 1986 [4], research on the possible role of IAPP in diabetes has focused on the amyloid-forming propensity of the peptide. Clearly, amyloid depositions in islets in Type II diabetes, either as a primary or a secondary event, is not beneficial for islet function. The mechanisms of islet-amyloid formation were recently reviewed elsewhere [48] and will not be discussed here. In addition, some early studies [37] suggested that hormonal actions of IAPP in peripheral, insulin-responsive tissues, could interfere with glucose disposal. Although there is some evidence that these actions are receptor-mediated [49], the mechanisms are still to be fully clarified and, thus, their relevance to the development of metabolic perturbations in Type II diabetes is not clear. Here, we would like to explore the notion that IAPP in islets could play a part in the development of Type II diabetes through mechanisms other than amyloid formation.

Accepting a teleological perspective on evolution, that IAPP is phylogenetically well conserved [14] strongly suggests it plays a part in biology. From the same perspective, it is more difficult to reconcile the sequence variation in some species, including humans, that create an amyloidogenic motif in $IAPP_{25–28}$ [50], with favourable selection in evolution. Conceivably, until the unanticipated recent ascent of Type II diabetes in western(ised) populations, they are of little significance in evolutionary terms. Islet amyloid formation and its diabetogenic potential aside, can IAPP in beta cells play a part in the development of Type II diabetes? There are several effects attributed to the peptide that could be harmful to or protective for beta cells in the development of this disease. As reviewed above, on the one hand, most data available at this point indicate that IAPP inhibits insulin secretion; such inhibition could add to the perturbed insulin response typical of Type II diabetes [51]. On the other hand, IAPP has been shown to hyperpolarise beta-cell membranes [25], an effect that could protect beta cells from exhaustion when demands for insulin secretion are high, such as in peripheral insulin resistance. It should also be borne in mind that IAPP has a neuropeptide-like structure; the sequence of IAPP shares about 50 % identity with that of CGRPs; [5, 6], the most ubiquitously expressed neuropeptides in primary sensory neurons. Here, IAPP is expressed in a subpopulation of CGRP-containing sensory neurons [12] where it seems to act as a prosensory neuropeptide [52]. Some neuropeptides are known to enhance neuronal survival [53] and, perhaps, IAPP could exert similar effects on the beta cell, a cell type which has several neuronal-like features [54].

Overexpression of IAPP in experimental diabetes

Early studies pointed to an up-regulation of IAPP expression compared with insulin expression in experimental rodent diabetes. Initially it was found that IAPP mRNA expression is fourfold higher than insulin mRNA expression after dexamethasone treatment [55]. In addition, after streptozotocin-induced diabetes in rats, IAPP mRNA expression was found to be sixfold less reduced than that of insulin mRNA [55]. Although insulin expression in absolute terms is likely to vastly exceed that of IAPP under the examined conditions [56–58], the ratio of IAPP mRNA to insulin mRNA is increased. Given that there could be a balance of the actions of IAPP with those of insulin, the ratio of IAPP and insulin expression is perhaps a more appropriate index than the absolute level of expression. It was later confirmed that the observed alterations in the ratio of hormone expression also translate into hormone production and release; the ratio of IAPP to insulin release is increased from the perfused pancreas of dexamethasone-treated or streptozotocin-treated rats [59, 60] as well as in spontaneously diabetic Zucker rats [61]. Furthermore, IAPP content is either more increased or less reduced than insulin content in dexamethasone-treated or streptozotocin-treated rats, respectively [62, 63].

Given the expression of IAPP in non-beta cells [64] as well as the proliferative effects of glucocorticoids in islets [65], we extended the studies on IAPP

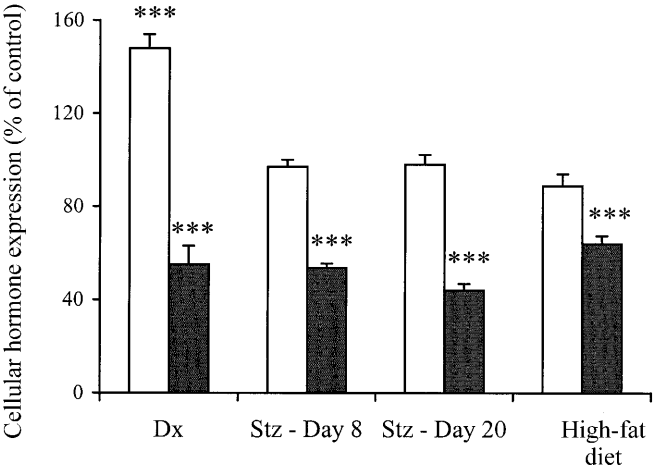

Fig. 2. Cellular IAPP (□) and insulin mRNA (■) expression determined under different metabolically challenging circumstances in rats (except high-fat diet), using quantitative in situ hybridisation; mRNA expressions graphed are the mean mRNA expressions in the islet cells which express the respective hormone and thus do not take into account change in cell number. Dx, dexamethasone treatment (2 mg/kg) daily for 12 days; Stz, streptozotocin treatment (70 mg/kg) at day 0 followed by mRNA analysis at day 8 or 20; high-fat diet, 58% fat on a caloric base compared with 11% in the control diet and given to C57BL/6J mice for 48 weeks. ***$p < 0.001$ compared with untreated controls. Data from [62, 67, 68, 83]

and insulin expression in experimental diabetes using cellular analysis (Fig. 2). In situ hybridisation to IAPP and insulin mRNA confirmed that IAPP expression is more highly up-regulated than that of insulin after dexamethasone treatment for 12 days [55, 62]. Whereas IAPP mRNA expression is increased at the cellular level, insulin mRNA expression is actually down regulated by dexamethasone treatment. In accordance with previous studies [65], we noticed a marked hyperplasia and hypertrophy of islet cells in the treated rats; this increase in islet mass explains that although insulin mRNA expression at the cellular level decreases, the total islet expression of insulin still increases twofold.

In streptozotocin-diabetic rats, IAPP and insulin mRNA expressions are reduced to 24 and 15% of that in controls at day 7 [66], as examined by in situ hybridisation, thereby confirming the initial observations [55]. The phenomenon is neither species-specific nor agent-specific because a similar regulation was observed in alloxan-treated mice [66]. Moreover, the differential regulation of IAPP and insulin expression seems not to be a transient phenomenon because in rats challenged with a high dose of streptozotocin (70 mg/kg) IAPP expression compared with controls was twofold higher than that of insulin also after three weeks [67, 68]. The differential regulation of the two hormones was further underscored by cellular IAPP mRNA expression being similar to those in controls whereas the insulin mRNA expression decreases to between 44 and 59% of that in controls at both 1 and 3 weeks after streptozotocin treatment, respectively [67, 68].

Potential mechanisms of differential expression of IAPP and insulin in diabetes

In addition to the regulation in experimental diabetes described above, other observations also indicate that IAPP and insulin expression are not always co-ordinately regulated. During embryonic development of the rat pancreas, IAPP is expressed in islet cells which lack insulin [64]. Moreover, when the pancreatic transcription factor Nkx 2.2 is genetically removed [69], islet cells remain which express IAPP while insulin is lacking. In adult animals, IAPP expression occurs in pancreatic and gastrointestinal somatostatin (D)-cells [64] as well as in primary sensory neurons [12], cell types which all clearly lack insulin expression. It should be noted that in response to changes in ambient glucose concentrations in vivo, islet IAPP and insulin mRNA expressions appear to be regulated in parallel [70, 71].

After dexamethasone treatment, alterations in beta-cell gene expression presumably occur in response to the metabolic perturbations but the steroid could act also by a direct effect on the beta cell because rat beta cells express glucocorticoid receptors [72]. The differential effect on IAPP and insulin gene expression could be explained by the presence of a negative regulatory element in the insulin gene promoter, which is lacking in the IAPP gene promoter and which binds dexamethasone [73]. Moreover, when a dose of dexamethasone and a duration of treatment are chosen to avoid the islet proliferative effects [65], no dissociation of IAPP and insulin expression occurs.

In the studies with impaired beta-cell mass, the mechanistic role of glucose in the dissociated expression of IAPP and insulin deserves attention. In streptozotocin-diabetic rats, insulin treatment prevents regeneration of beta cells and insulin mRNA expression is lower than in untreated, more highly hyperglycaemic, controls [74]. These findings were confirmed by us [68] but, in contrast, IAPP expression was not changed by the insulin treatment. Moreover, it has previously been shown that although insulin mRNA expression is down regulated, IAPP mRNA expression is not changed in a hyperinsulinaemic clamp in rats [75]. This lack of effect of insulin on IAPP expression [68, 75] also raises the possibility that a metabolic perturbation in diabetes, other than hyperglycaemia, could be responsible for the dissociation in IAPP and insulin expression. Lipid abnormalities have recently been increasingly implicated in beta-cell dysfunction [76]. Notably, a transgenic mouse strain expressing the human amyloid-forming IAPP species in beta

cells develops hyperglycaemia and islet amyloid when given a high-fat diet [77]. Although islet amyloid formation in other similar transgenic mouse strains seems to depend on additional factors (e.g. genetic background [78, 79], dose of the transgene [80], or induction of insulin resistance by treatment with steroids and growth hormone [81]), this raises the possibility that lipid perturbations are involved in the dissociation of IAPP and insulin expression. A 4.5-fold increase in the ratio of circulating fasting plasma IAPP to insulin occurs in NMRI mice fed a high-fat diet for 6 months [82]. In this study the mice did not, however, appear to be insulin-resistant because fasting insulin concentrations were similar to those in mice fed a normal diet [unpublised data]. To further investigate this issue, we examined IAPP expression at the cellular level in obesity-prone C57BL/6J mice fed a high-fat diet for 48 weeks [83]; these mice are not overtly hyperglycaemic but have lipid abnormalities, such as increased circulating concentrations of triglycerides, free fatty acids and leptin. Under these conditions, cellular IAPP expression is not altered whereas that of insulin is down-regulated. Notably, the increased ratio of IAPP expression to that of insulin does not translate into non-fasted circulating hormone concentrations; although circulating IAPP concentrations rose by 60%, insulin concentrations were threefold increased. A similar decrease in the ratio of circulating IAPP to insulin occurs in the severely insulin resistant *ob*/*ob* mouse [84]. The reason for the discrepancy between the ratio of islet hormone expression and the ratio of circulating hormone expression is not clear at this point but could relate to the kinetics of hormone elimination from the circulation being different for IAPP and insulin [85, 86].

IAPP-deficient mice are more susceptible to alloxan-induced diabetes

Although it is clear that IAPP is overexpressed compared with insulin in relative terms in experimental forms of diabetes, the role of such regulation is not clear. As discussed above, there are scenarios in which both beneficial and adverse effects can occur. To address this issue, we used male *IAPP* null mutant mice to investigate whether the lack of IAPP would affect the development of experimental diabetes [87]. After 35 days, alloxan-treated *IAPP* null mutants were more severely diabetic. This is due to a greater impairment of islet function, reflected by a more pronounced hyperglycaemia and hypoinsulinaemia (Fig. 3). Beta-cell mass and the number of beta cell-containing islets are more reduced in the mutants. Furthermore, the *IAPP* mutants have exaggerated beta-cell dysfunction because in their remaining beta cells insulin mRNA expression is more impaired and the localisation of glucose transporter-

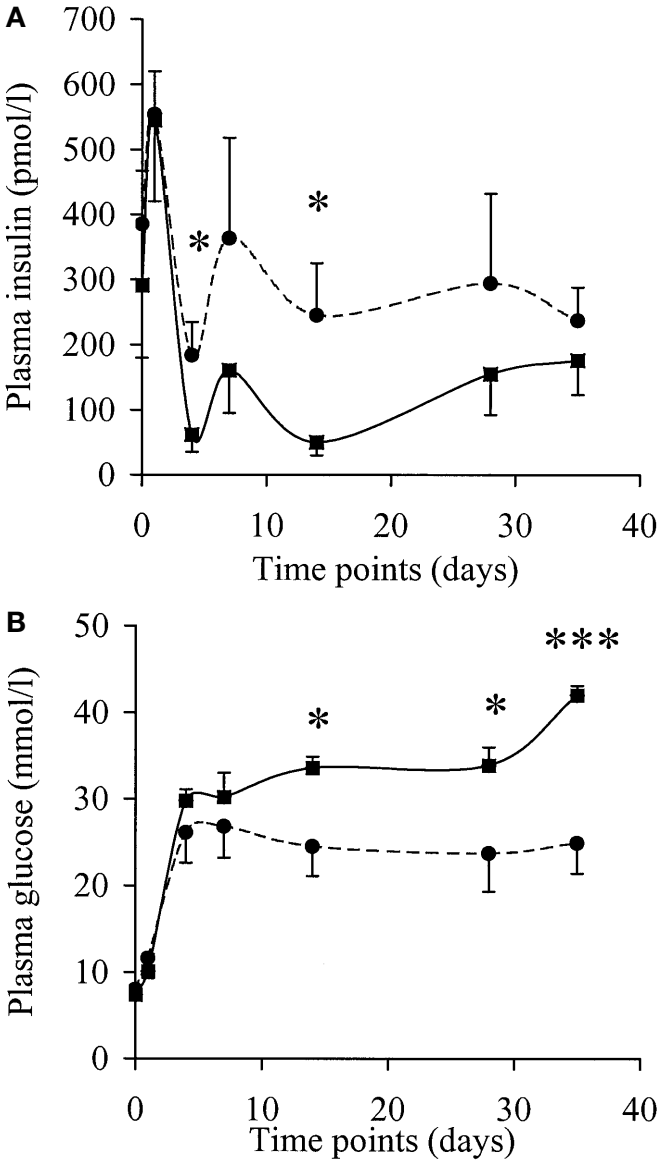

Fig. 3 A, B. Plasma insulin (**A**) and glucose (**B**) concentrations in wild-type (--●--) and *IAPP* null mutant (–■–) mice treated with 70 mg/kg alloxan i.v. The *IAPP* null mutant mice are more severely diabetic, having more pronounced hypoinsulinaemia and hyperglycaemia during the study period of 35 days. $*p < 0.05$ and $***p < 0.001$ compared with wild-type mice at each time point. Data from [87]

2 is perturbed. Thus, the lack of IAPP allows exaggerated beta-cell cytotoxic actions of alloxan, suggesting that there could be beneficial features of IAPP actions in situations of beta-cell damage. Notably, the reverse phenotype is apparent in non-obese diabetic (NOD) mice with a targeted expression of CGRP to beta cells; this manoeuvre either prevents diabetes or decreases its incidence in male and female mice, respectively [88]. The rationale here is that IAPP and CGRP exert their effects through similar receptors, a phenomenon which has previously been shown repeatedly [44].

Several putative mechanisms could underlie the aggravated diabetes in the IAPP-deficient mice. It was suggested that a local immunomodulatory action of CGRP prevents diabetes in the CGRP transgenic NOD mice [88]. Such immunomodulation could involve control of islet circulation and here IAPP could have a role because it increases the fractional blood flow through islets [89]. Lack of such a blood flow increase in the *IAPP* mutants could impair islet regeneration after the alloxan insult and IAPP could act as a growth factor in islets; in cultured renal cells IAPP promotes growth [90]. The absence of an IAPP-mediated promotion of islet growth/regeneration could explain the persistent impairment of islet function. Moreover, IAPP inhibits insulin secretion in patch-clamped beta cells by hyperpolarising the plasma membrane [25] and could therefore limit prolonged depolarisation of beta cells with ensuing increase of intracellular $Ca2^{+}$-concentrations. If such actions protect beta cells from toxic effects of hyperglycaemia, lack of IAPP could aggravate beta-cell damage in diabetes.

Concluding remarks and future perspectives

During the past decade, IAPP has emerged as one of several new players in the complex control of insulin secretion and beta-cell function. Overexpression or genetic ablation of IAPP in mice and the phenotypes resulting therefrom show that IAPP is a physiological inhibitor of glucose-stimulated insulin release in rodents. Although the precise mechanism(s) of this action of IAPP are not clear, at this point an autocrine/paracrine mechanism is the most likely one. The recent identification of IAPP receptors as heterodimers between CTRs and different RAMP species provides a means by which putative IAPP target tissues can be identified. In diabetes, amyloid-formation aside, the established overexpression of IAPP raises the possibility that it arises as a protective mechanism in islets. The aggravated diabetic phenotype in *IAPP* null mutants supports this notion and several beneficial aspects of IAPP actions could form the basis of such a protective function. Because impaired beta-cell function and mass are characteristic features of human Type II diabetes and of Type II diabetes-like conditions in several rodent models, the implications of a protective role of IAPP in islets deserve further attention. Bearing the islet amyloid-forming propensity of IAPP in mind, it could be premature to proclaim IAPP a "friend" in the islets. Nevertheless, considering the beneficial features of IAPP actions discussed here, a more balanced view of IAPP in islets could be justified.

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