Lack of Islet Amyloid Polypeptide Regulation of Insulin Biosynthesis or Secretion in Normal Rat Islets

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We examined the effects of rat islet amyloid polypeptide (IAPP) on insulin biosynthesis and secretion by isolated rat islets of Langerhans. Culture of islets for 24 h in the presence of 10^{-6} M IAPP and 5.5 mM glucose had no effect on insulin mRNA levels. Similarly, the rates of proinsulin biosynthesis were not altered in islets incubated in 10^{-4}–10^{-9} M IAPP and 5.5 mM glucose, nor was the rate of conversion of proinsulin to insulin changed at 10^{-6} M IAPP. Addition of 10^{-5} M IAPP to islets incubated in 11 mM glucose decreased the fractional insulin secretion rate; however, the secretion of newly synthesized proinsulin and insulin was not affected. These data indicate that it is unlikely that IAPP is a physiologically relevant modulator of insulin biosynthesis or secretion.
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Islet amyloid polypeptide (IAPP) is a neuroendocrine peptide structurally related to calcitonin gene–related polypeptide (CGRP) and is a major constituent of islet amyloid deposits in pancreases of diabetic humans and cats and also in some human insulinomas (1–5). One study recently demonstrated that the predicted amino acid sequences of the IAPP domains in several mammalian IAPP precursors, including those of the cat, rat, mouse, and guinea pig, are highly conserved, consistent with the possibility that IAPP is a peptide hormone of the islets of Langerhans (6). Evidence that IAPP is colocalized with insulin in islet secretory granules (7) and is probably secreted along with insulin and C-peptide (8,9) further supports this notion and suggests that IAPP may have significant physiological effects in the body.

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CGRP and IAPP are both 37–amino acid polypeptides that appear to be members of a superfamily of neuroregulatory peptides that also includes calcitonin (10) CGRP has been reported to be widely distributed in the peripheral nervous system (11) and has several biological effects, including induction of vasodilation (12), inhibition of gastric acid secretion (13), and inhibition of insulin secretion (14,15,16). Because of its close structural resemblance to CGRP, IAPP appears to have similar peripheral biological functions (17) and may also have direct effects on some pancreatic islet functions.

The presence of IAPP as the major protein in amyloid deposits found in the islets of patients with non-insulin-dependent diabetes mellitus (NIDDM) has lead to the suggestion that IAPP secretion and/or amyloid deposition may play a primary role in the pathogenesis of diabetes (14,18,19). It is therefore important to study the possible effects of IAPP on pancreatic islet functions. Accordingly, we examined its effects on preproinsulin gene expression, proinsulin biosynthesis and conversion to insulin, and insulin secretion in normal rat islets.

RESEARCH DESIGN AND METHODS

Rat IAPP (amidated) was purchased from Peninsula (Belmont, CA). Protein A-sepharose was from Pierce (Rockford, IL). A kit for nick translation and L-[3,4,5-^{3}H]leucine were from Amersham (Arlington Heights, IL). Nitrocellulose membranes were from Schleicher & Schuell (Keene, NH). [α-^{32}P]dCTP was purchased from Du Pont-NEN (Boston, MA).

Islets of Langerhans were isolated as described elsewhere from fed male Sprague-Dawley rats (20). Islets were incubated with RPMI-1640 medium containing 10% (vol/vol) fetal calf serum (FGS) at 37°C in 95% air/5% CO_2 under the various conditions described in Figs. 1–4.

RNA extraction and Northern blotting were done as follows. Four hundred islets were incubated for 24 h in the presence or absence of 10^{-6} M IAPP at 5.5 mM glucose. Total RNA was extracted with the guanidine thiocyanate procedure (21). All of the extracted RNA was applied onto a

1.5% agarose/0.66 M formaldehyde gel, blotted onto a nitrocellulose filter, and hybridized with nick-translated rat insulin cDNA. Filters were washed under high-stringency conditions (0.1 × SSC, 0.1% sodium dodecyl sulfate, 65°C) and autoradiographed with intensifying screens.

Groups of 30–60 islets in 50 μl of RPMI-1640/10% dialyzed FCS medium without leucine were labeled with 200 μCi/ml[^{3}H]leucine for 30 min in the glucose concentration described in each figure in the presence or absence of IAPP. For investigation of proinsulin conversion to insulin or secretion of labeled proinsulin and insulin, islets were then washed and chase incubated for 60 min in RPMI-1640 medium.

Immunopurification of insulin and proinsulin was performed as follows. Islets were lysed by the freeze and thaw method, lyophilized, and then dissolved in 100 μl H_2O. The cell lysate (10 μl) and the supernatant after the chase incubation were immunoprecipitated with excess guinea pig anti-insulin antibody (1 μl of undiluted antibody GP-3) and pelleted with protein A-sepharose after prior absorption with normal guinea pig serum. Total protein synthesis was determined after precipitation of islet lysate in 10% trichloroacetic acid. Proinsulin and insulin were separated by Bio-Gel P-30 column chromatography as described elsewhere (22,23). Radioactivity was determined by liquid-scintillation counting.

Insulin in medium or islets was assayed as immunoreactive insulin with rat insulin standards by an enzyme-linked immunosorbent assay (24) with minor modifications. Fractional rates of secretion were calculated by assigning the value of 100% to the sum of all islets and secreted hormone. Measurements of relative radioactivity in proinsulin and insulin were corrected for the loss of C-peptide by multiplying radioactivity eluting to the insulin peak by 1⅛ (22). All values are reported as means ± SE, and differences were assessed by paired Student's *t* test. The average of experiments conducted in duplicate or triplicate on a given day was taken as $n = 1$.

RESULTS

The levels of preproinsulin mRNA in islets cultured with or without 10^{-6} M IAPP for 24 h at 5.5 mM glucose was measured by Northern blot analysis. No effect of IAPP on preproinsulin transcription could be detected by densitometric analysis of blots from three experiments (data not shown).

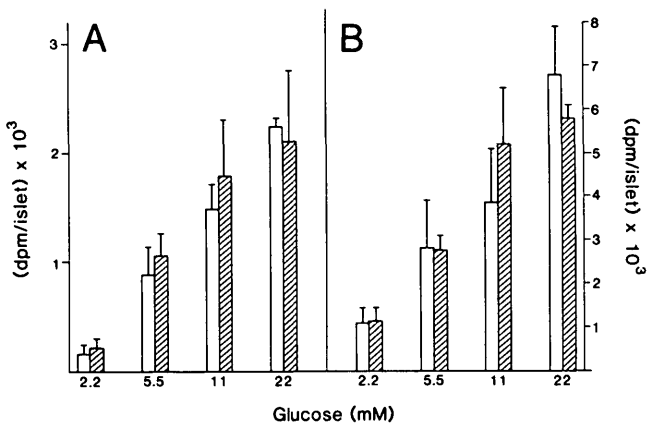

FIG. 1. Effect of islet amyloid polypeptide (IAPP; *hatched bars*) on both proinsulin (*A*) and total protein (*B*) biosynthesis in 2.2, 5.5, 11.0, and 22.0 mM of glucose. *Open bars*, IAPP$^-$. Groups of islets were incubated with 10^{-6} M IAPP in 2.2, 5.5, 11.0, and 22.0 mM of glucose for 3 h at 37°C and then pulse labeled with [^{3}H]leucine under same conditions. Proinsulin and total protein biosynthesis were measured as described in RESEARCH DESIGN AND METHODS ($n = 3$).

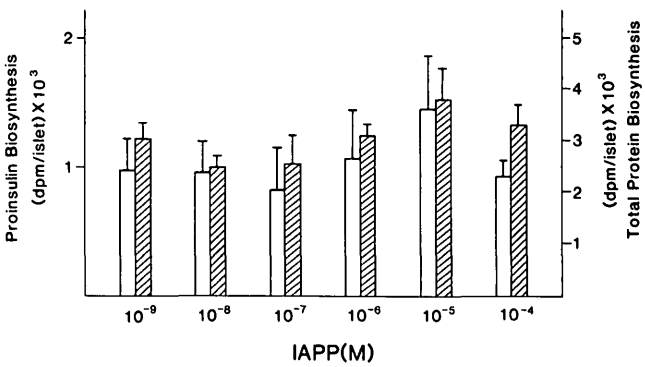

FIG. 2. Effect of concentration of islet amyloid polypeptide (IAPP) on both proinsulin (*hatched bars*) and total protein (*open bars*) biosynthesis in 5.5 mM glucose. Groups of islets were incubated with 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, and 10^{-9} M IAPP in 5.5 mM glucose for 3 h at 37°C and then labeled with [^{3}H]leucine under same conditions. Islets were then processed as described in Fig. 1 ($n = 3$).

The effects of 10^{-6} M IAPP on the rate of proinsulin biosynthesis were tested. After 3 h incubation of islets with 10^{-6} M IAPP at several concentrations of glucose, labeling of either total extractable protein or proinsulin was unaffected by IAPP (Fig. 1). Extending the incubation period to 24 h in 5.5 mM glucose with 10^{-5} M IAPP also did not affect proinsulin synthesis (data not shown).

The biosynthetic effects of IAPP in concentrations ranging from 10^{-4} to 10^{-9} M were also tested after 3 h preincubation with IAPP in 5.5 mM glucose. However, no significant effects on the synthesis of either total protein or proinsulin were found (Fig. 2). Prior exposure to high glucose or cycloheximide is known to accelerate or inhibit proinsulin conversion to insulin in islets (25,26). However, pretreatment of islets with 10^{-6} M IAPP had no effect on the rate of proinsulin conversion (~55% proinsulin after 60-min chase incubation).

IAPP (10^{-5} M) significantly inhibited the secretion of immunoreactive insulin from islets incubated in 11 mM glucose, although it did not affect secretion of newly synthesized insulin (Fig. 3). At more moderate doses (10^{-6}–10^{-9} M), no significant effects of IAPP on either immunoreactive or newly synthesized insulin secretion were observed (data not shown). The effect of IAPP on glucose-stimulated insulin secretion was also evaluated in the isolated perfused rat pancreas. At 10^{-6} M, no significant acute effects were observed (Fig. 4).

DISCUSSION

This study was performed to test the hypothesis that IAPP may play a role in the regulation of some functions of the pancreatic β-cells. We tested the effects of IAPP in vitro on insulin-gene expression, proinsulin biosynthesis and its conversion to insulin, and the secretion of newly synthesized insulin. We found that 10^{-6} M IAPP did not affect the level of preproinsulin mRNA as measured by Northern blot analysis. No effects were observed at 10^{-5} M IAPP.

Initial experiments examining insulin biosynthesis were carried out at 10^{-6} M IAPP in the presence of 2.2, 5.5, 11.0, and 22.0 mM medium glucose concentrations, but no effects on proinsulin biosynthesis were seen. To eliminate the pos-

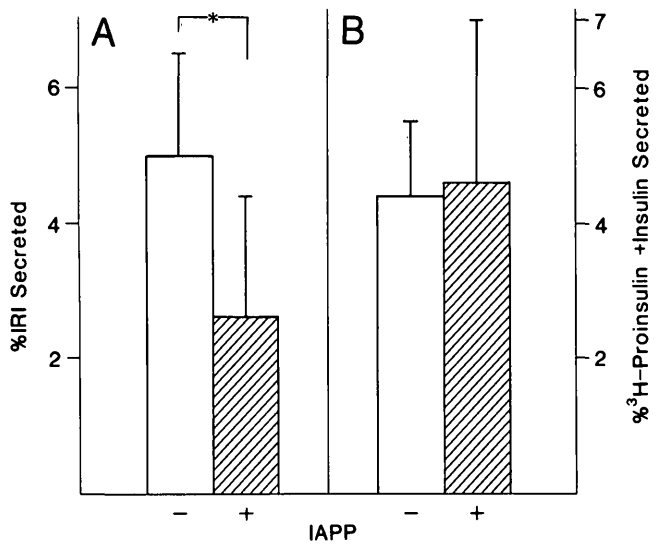

FIG. 3. Effect of islet amyloid polypeptide (IAPP) on secretion of both immunoreactive insulin (IRI; *A*) and newly synthesized proinsulin and insulin (*B*). For measurement of IRI, islets were incubated in RPMI-1640/10% dialyzed fetal calf serum in presence (*hatched bars*) or absence (*open bars*) of 10^{-5} M IAPP in 11 mM glucose for 1 h at 37°C. Supernatant was separated from islets and IRI was assayed as described in RESEARCH DESIGN AND METHODS. For newly synthesized insulin secretion, islets were incubated in presence or absence of 10^{-5} M IAPP in 11 mM glucose for 3 h at 37°C, labeled with [^{3}H]leucine for 30 min, and chase incubated for 60 min. Immunopurification of proinsulin and insulin from supernatant and islets was carried out as described in RESEARCH DESIGN AND METHODS. Fractional secretory rates were calculated with sum of all insulin secreted, and islet insulin was assigned value of 100%. *$P < 0.001$ by paired Student's *t* test.

sibilities that IAPP might become bound to proteins in FCS or be degraded, we used Hanks' buffer with crystalline bovine serum albumin instead of RPMI-1640/10% FCS for both incubation and labeling. However, no differences were apparent between the two media (data not shown). Either increasing the dose of IAPP up to 10^{-4} M or extending the prior incubation period with IAPP to 24 h also had no observable effect on proinsulin biosynthesis. Finally, to test the hypothesis that hyperproinsulinemia in NIDDM may be somehow related to IAPP (27), we examined its effect on proinsulin conversion but found none.

Synthetic IAPP has been reported to inhibit insulin secretion, although the dose (10^{-5} M) required for this effect was extremely high (28). The predicted amino acid sequences of several IAPP precursors indicates that the COOH-terminal tyrosine of IAPP is likely to be amidated (6,29). We therefore studied the effects of the amidated form of rat IAPP on insulin secretion. We also found that a high level of amidated IAPP (10^{-5} M) was required to inhibit glucose-stimulated insulin secretion. Indeed, in our study, the inhibitory effect of IAPP was not as marked as that previously reported with the non-amidated peptide (28). Furthermore, this high dose did not affect the secretion of newly synthesized insulin. Also, in the isolated perfused pancreas, which is usually more sensitive to inhibitors, no acute effects of IAPP (10^{-6} M) on insulin secretion were observed. Thus, the observed effects of IAPP on insulin secretion in islets are likely to be pharmacological rather than physiological.

As mentioned previously, it seems possible that IAPP may share some actions of CGRP because of its structural similarity. The effects of CGRP appear to be mediated via cAMP (11), which regulates insulin secretion in islets (30,31), and preproinsulin gene transcription (32). Surprisingly, our data have not indicated that IAPP has any direct actions on β-cells that might be attributable to cAMP generation, suggesting that there may be no IAPP receptors in β-cells. The inhibition of insulin secretion, however, might be mediated via structurally related CGRP receptors with which IAPP may cross-react or via interactions with other islet cells (α- or δ-cells).

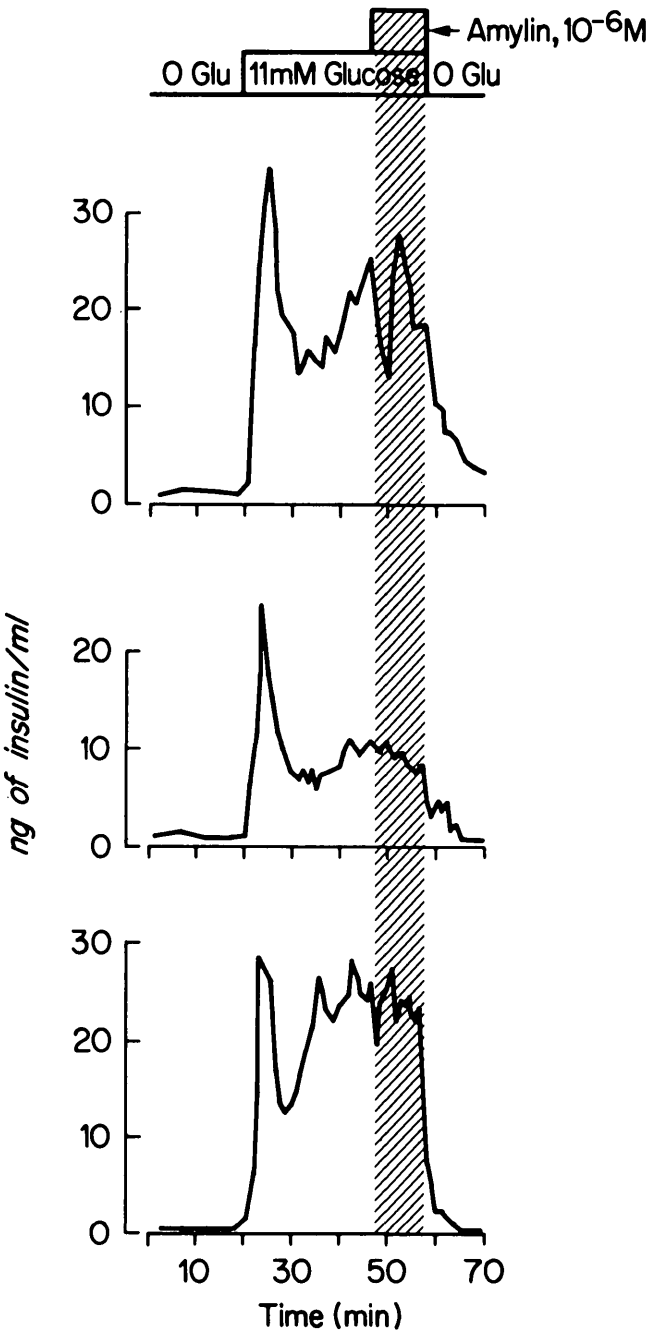

FIG. 4. Lack of effect of 10^{-6} M islet amyloid polypeptide (amylin; *hatched area*) on glucose-induced insulin secretion in isolated perfused rat pancreas. Pancreases with adjacent intact stomach, spleen, and partial duodenum were removed from 350-g, fed, male Long-Evans rats and perfused as previously described (36). Standard buffered dextran–bovine serum albumin medium (37) was perfused through celiac artery at flow rate of 2 ml/min, and effluent fractions were collected from portal vein at 1-min intervals and assayed for insulin.

Other studies have indicated that levels of IAPP in islets are ~1–2% of those of insulin (33–35). If this is so, then the levels of IAPP that could be expected to occur under normal physiological conditions are unlikely to exceed 10^{-8} M. Thus, these experiments on isolated islets revealed no direct inhibitory effects of IAPP on pancreatic β-cell functions at physiologically relevant concentrations. These experiments do not rule out the possibility that IAPP secreted along with insulin may act locally on islet blood vessels to enhance blood flow and thereby to augment insulin delivery. IAPP-associated amyloid deposition may eventually cause a block in glucose-stimulated insulin secretion that could contribute to the development of NIDDM (33).

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Activation of Adenylate Cyclase by Islet Amyloid Polypeptide With COOH-Terminal Amide via Calcitonin Gene–Related Peptide Receptors on Rat Liver Plasma Membranes

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Both human and rat islet amyloid polypeptide with COOH-terminal amide (IAPP-NH_2) dose-dependently displaced the specific binding of ^{125}I-labeled [Tyr^0] rat α-calcitonin gene–related peptide (CGRP) to rat liver plasma membranes, whereas human IAPP (IAPP-COOH) had no effect. Conversely, human or rat IAPP-NH_2 but not human IAPP-COOH evoked dose-dependent activation of adenylate cyclase in the membranes, and these effects were significantly inhibited by the CGRP-receptor antagonist human CGRP-I(8-37). Moreover, the dose of human or rat IAPP-NH_2 necessary for producing half-maximal activation of adenylate cyclase was comparable with that for producing a half-maximal inhibition of the label binding. Thus, IAPP-NH_2 but not IAPP-COOH appears to induce adenylate cyclase activation via CGRP receptors on rat liver plasma membranes. *Diabetes* 39:875–77, 1990

Amyloid deposits are often found in pancreatic islets of patients with non-insulin-dependent diabetes mellitus and in insulinomas (1). Two groups have isolated and sequenced identical amyloid proteins from human insulinoma and from the pancreas of diabetic patients, and these have been designated islet amyloid polypeptide (IAPP) and amylin respectively (2,3). IAPP has also been demonstrated by immunocytochemistry in the secretory granules of β-cells (4). Moreover, Sanke et al. (5) identified cDNA clones encoding IAPP from human insulinoma and predicted that the IAPP precursor possesses a typical signal peptide followed by a small prohormonelike sequence. These findings suggest that IAPP is secreted from pancreatic islets and has important hormonal significance.

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However, in previous studies, we identified specific receptors for calcitonin gene–related peptide (CGRP) linked to adenylate cyclase activation on rat liver plasma membranes, suggesting a role for CGRP in glucose metabolism in the liver (6,7). Interestingly, human IAPP, a 37–amino acid polypeptide with cysteine residues at positions 2 and 7, shows 43 and 46% amino acid sequence homology with human CGRP-I and CGRP-II, respectively, whereas rat IAPP possesses 49 and 46% homology with rat α-CGRP and β-CGRP, respectively.

This study was therefore undertaken to examine whether IAPP may act on CGRP receptors present on the rat liver plasma membranes to activate adenylate cyclase. Furthermore, because the predicted sequence of the IAPP precursor suggests that the secreted form of IAPP may be carboxyamidated (8), we also compared the effects of IAPP with COOH-terminal amide (IAPP-NH_2) with those of human IAPP (IAPP-COOH) on CGRP receptors.

RESEARCH DESIGN AND METHODS

Synthetic [Tyr^0]α-CGRP, β-CGRP, and rat IAPP-NH_2 were purchased from Peninsula (Belmont, CA). Synthetic human CGRP-I(8-37), the nonamidated form of rat α-CGRP (α-CGRP-COOH), IAPP-COOH, and human IAPP-NH_2 were synthesized via an automatic solid-phase synthesizer (430-A peptide synthesizer, Applied Biosystems). ^{125}I-labeled [Tyr^0] rat α-CGRP (2000 Ci/mmol) was prepared as described previously (9). A cyclic AMP (cAMP) assay kit was obtained from Yamasa Shoyu (Choshi, Japan).

Rat liver plasma membranes were prepared from adult male Sprague-Dawley rats by the method of Pilkis et al. (10) with slight modification (11). The fraction collected at the 42.5–48.2% sucrose interface was removed and washed with 10 mM HEPES buffer (pH 7.4) containing 120 mM NaCl, 4.7 mM KCl, 5 mM $MgCl_2$, 1 mM EGTA, 1 mg/ml bacitracin, and 5 mg/ml bovine serum albumin (BSA). One-half milliliter of this membrane suspension (200 μg tissue protein) containing ^{125}I-[Tyr^0]α-CGRP (25 pM) was incubated at 24°C for 60 min with or without various unlabeled peptides. Bound

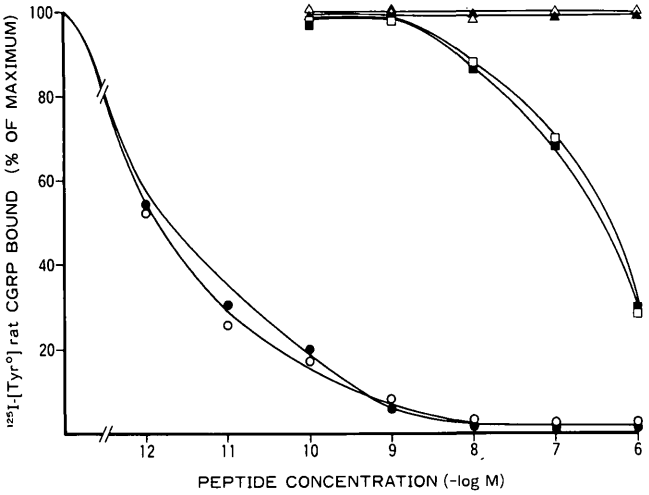

FIG. 1. Effects of various peptides on specific binding of ^{125}I-labeled [Tyr^0]α-calcitonin gene–related peptide (CGRP) to rat liver plasma membranes. Rat liver plasma membranes were incubated for 60 min at 24°C with 25 pM ^{125}I-[Tyr^0]α-CGRP in presence or absence of α-CGRP (●), β-CGRP (○), human islet amyloid polypeptide (IAPP)-NH_2 (■), rat IAPP-NH_2 (□), α-CGRP-COOH (▲), or IAPP-COOH (△). Data are expressed as percentages of maximal specific binding in absence of unlabeled peptides. Values are means of 5 separate experiments differing by 5%. Each experiment was done in duplicate.

and free hormones were separated by centrifugation at 10,000 × *g* for 15 min, and radioactivity bound to the pellets was counted by an auto-γ-counter. Nonspecific binding was assessed as the fraction of label that remained bound in the presence of 10^{-6} M [Tyr^0]α-CGRP.

The adenylate cyclase activity in rat liver plasma membranes was determined by measuring the synthesis of cAMP from nonradioactive ATP (12). Membranes (10 μg protein) were incubated for 10 min at 37°C in 100 μg Tris buffer (25 mM, pH 7.6) containing 1 mM EGTA, 1 mg/ml BSA, 100 mM NaCl, 100 μM GTP, 1 mM ATP, 5 mM $MgCl_2$, 1 mM isobutylmethylxanthine, and 1 mM aprotinin with or without various peptides unless otherwise indicated. The buffer also contained 5 mM phosphocreatine and 50 U/ml creatine phosphokinase as the ATP-regenerating system. After incubation, the reactions were terminated by adding 40 μl 0.33 M $ZnSO_4$ and 40 μl 0.5 M Na_2CO_3. The precipitate was centrifuged at 10,000 × *g* for 5 min, and cAMP levels in the supernatant were measured by specific radioimmunoassay (13). Adenylate cyclase activity was expressed as picomoles of cAMP synthesized with 1 μg membrane protein during 1 min of incubation.

RESULTS AND DISCUSSION

The specific binding of ^{125}I-[Tyr^0]α-CGRP to rat liver plasma membranes was dose-dependently displaced not only by α-CGRP and β-CGRP but also by human and rat IAPP-NH_2, although the doses of human and rat IAPP-NH_2 necessary for producing half-maximal inhibition (IC_{50}) of label binding were 1 × 10^5 times higher than those of rat CGRPs (Fig. 1; Table 1). On the other hand, all of α-CGRP, β-CGRP, and human and rat IAPP-NH_2 also evoked dose-dependent activation of adenylate cyclase in rat liver plasma membranes. Moreover, the doses of these peptides necessary for producing half-maximal effects (EC_{50}) on adenylate cyclase activation were comparable to their respective IC_{50} values in the binding study (Fig. 2; Table 1). Because we did not examine the binding of the labeled IAPP-NH_2 to rat liver membranes in this study, it might be possible to speculate from these data that part of the ^{125}I-[Tyr^0]α-CGRP was bound to the specific receptors for IAPP-NH_2 and that this binding was displaced by unlabeled IAPP-NH_2. However, this seems unlikely because our previous binding study revealed a single class of binding site for ^{125}I-[Tyr^0]α-CGRP on rat liver plasma membranes, excluding the possibility of cross-reaction of this label with other receptors (6). Furthermore, this study also demonstrated that the activation of adenylate cyclase induced by both human and rat IAPP-NH_2 was significantly inhibited by the CGRP-receptor antagonist human CGRP-I(8-37) (14; Fig. 3). These data strongly suggest that IAPP-NH_2 activates adenylate cyclase by binding to CGRP receptors on rat liver plasma membranes. Indeed, IAPP-NH_2 is homologous with CGRPs not only at the NH_2-terminal but also at the COOH-terminal (3), and we have shown that the COOH-terminal portion of CGRP is important for binding to its receptors (6). However, because we did not examine the binding of labeled IAPP-NH_2 to rat liver membranes in this study, it cannot be ruled out that IAPP-NH_2 possesses its own specific receptors on liver plasma membranes that are not linked to adenylate cyclase activation.

In contrast to the effects of human or rat IAPP-NH_2, neither α-CGRP-COOH nor IAPP-COOH up to a concentration of 10^{-6} M had any effect on either the binding of ^{125}I-[Tyr^0]α-CGRP to or adenylate cyclase activity in rat liver plasma membranes in this study (Figs. 1 and 2). These data indicate that the COOH-terminal amide is essential for binding to CGRP receptors. In this regard, note that the secretory form of IAPP appears to be carboxyamidated (8), and moreover immunoreactive IAPP-NH_2 has been demonstrated in the human pancreas as well as in plasma, suggesting a hormonal significance of IAPP-NH_2 (15).

Leighton and Cooper (16) showed that, like CGRP, IAPP (amylin) inhibits insulin-induced glycogen synthesis in isolated soleus muscle preparations. Sowa et al. (17) demonstrated that human IAPP-NH_2 induces insulin resistance in vivo in dogs. On the other hand, using a specific antiserum against human IAPP-NH_2, Nakazato et al. (15) recently revealed that the concentration of IAPP-NH_2 in human plasma is 13.5 ± 4.8 pg/ml, which is clearly less than that eliciting a significant enhancement of adenylate cyclase in this study (10^{-8} M). However, Cooper et al. (18) reported that IAPP-

TABLE 1
Concentration of calcitonin gene–related peptide (CGRP) and islet amyloid polypeptide (IAPP) on rat liver plasma membrane

Peptides	IC_{50} (M)	EC_{50} (M)
Rat		
α-CGRP	9.2 × 10^{-12}	22.3 × 10^{-12}
β-CGRP	7.6 × 10^{-12}	25.9 × 10^{-12}
IAPP-NH_2	3.0 × 10^{-7}	3.8 × 10^{-7}
Human IAPP-NH_2	7.4 × 10^{-7}	8.2 × 10^{-7}

IC_{50} = concentration of unlabeled peptide required to inhibit the specific binding of ^{125}I-labeled [Tyr^0]α-CGRP by 50%. EC_{50} = concentration of peptide necessary for producing 50% of the increase in adenylate cyclase activity (from control) induced by a maximally effective concentration of α-CGRP (10^{-6} M). Values are means of 5 separate experiments. Each experiment was done in duplicate.

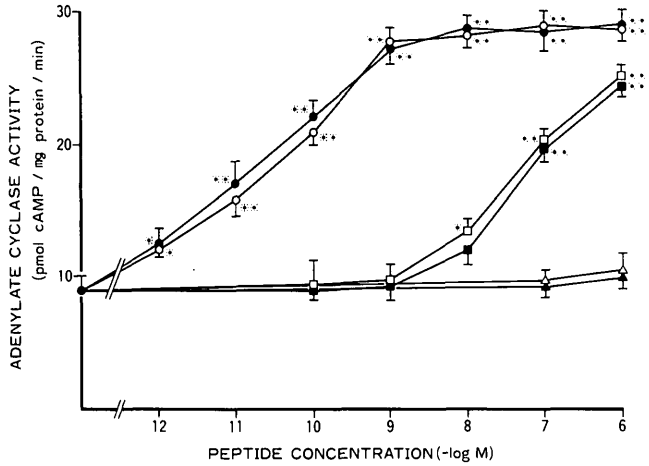

FIG. 2. Effects of various peptides on adenylate cyclase activation in rat liver plasma membranes. Rat liver plasma membranes were incubated with increasing concentrations of α-calcitonin gene–related peptide(CGRP) (●), β-CGRP (○), human islet amyloid polypeptide(IAPP)-NH_2 (■), rat IAPP-NH_2 (□), α-CGRP-COOH (▲), or IAPP-COOH (△) for 10 min at 37°C. Values are means ± SE of 5 separate experiments. Each experiment was done in duplicate. *$P < 0.05$, **$P < 0.01$, vs. control.

NH_2 may circulate in the blood of nondiabetic people at a concentration ~10 times higher than that of CGRP. Furthermore, in addition to the pancreas, IAPP-NH_2 has been demonstrated to be present in the stomach and upper intestine of humans, indicating that the concentration of IAPP-NH_2 in hepatic portal blood is much higher than that in peripheral blood (15). Therefore, the question of whether IAPP-NH_2 plays a role in the regulation of glucose metabolism via CGRP receptors in the liver under physiological conditions remains to be elucidated.

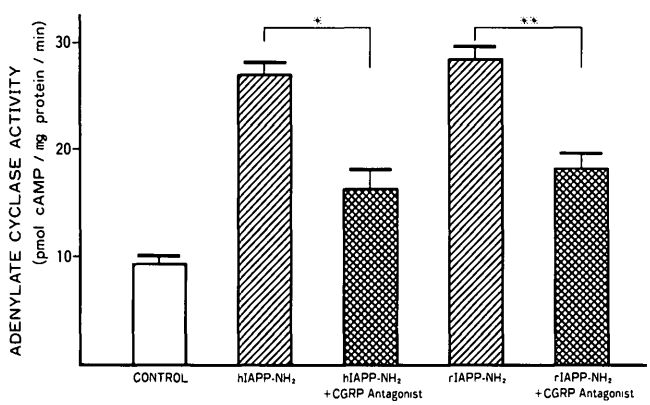

FIG. 3. Effect of human calcitonin gene–related polypeptide (CGRP)-I(8-37) (CGRP antagonist) on human (h) or rat (r) islet amyloid polypeptide (IAPP)-NH_2–induced activation of adenylate cyclase in rat liver plasma membranes. Rat liver plasma membranes were incubated with either 10^{-6} M human or rat IAPP-NH_2 alone or in combination with 10^{-5} M CGRP-I(8-37) for 10 min at 37°C. Values are means ± SE of 5 separate experiments. Each experiment was done in duplicate. *$P < 0.01$ vs. hIAPP-NH_2 alone. **$P < 0.01$ vs. rIAPP-NH_2 alone.

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