Original Article

CART Regulates Islet Hormone Secretion and Is Expressed in the β-Cells of Type 2 Diabetic Rats

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Cocaine- and amphetamine-regulated transcript (CART) is an anorexigenic peptide widely expressed in the central, peripheral, and enteric nervous systems. CART is also expressed in endocrine cells, including β-cells during rat development and δ-cells of adult rats. We examined the effect of CART 55–102 on islet hormone secretion, using INS-1(832/13) cells and isolated rat islets. In addition, islet CART expression was examined in two rat models of type 2 diabetes: Goto-Kakizaki (GK) rats and dexamethasone (DEX)-treated rats. At high glucose, CART potentiated cAMP-enhanced insulin secretion via the cAMP/protein kinase A-dependent pathway. In the absence of cAMP-elevating agents, CART was without effect on INS-1 cells but modestly inhibited secretion of insulin, glucagon, and somatostatin from isolated islets. CART was markedly upregulated in the β-cells of both diabetes models. Thus, in DEX-treated rats, islet CART mRNA expression, and the number of CART-immunoreactive β-cells were 10-fold higher than in control rats. In GK rats, the relative number of CART-expressing β-cells was 30-fold higher than in control rats. We conclude that CART is a regulator of islet hormone secretion and that CART is upregulated in the β-cells of type 2 diabetic rats. *Diabetes* 55:305–311, 2006

The islets harbor and are regulated by a variety of different neurohormonal peptides. Among such peptides are ghrelin, galanin, neuropeptide Y, peptide YY, and islet amyloid polypeptide (1–5). This illustrates a high complexity in the regulation of islet hormone secretion, and improved knowledge about islet peptides is important to more fully understand islet hormone regulation. The neuropeptide cocaine- and amphetamine-regulated transcript (CART) is highly expressed in the brain (6–12) and exhibits anorexigenic properties (13,14) (rev. in 15). CART is also expressed in the peripheral nervous system, including enteric (16,17) and pancreatic (18) neurons. Further, CART is expressed in neuroendocrine cells, including rat islet δ-cells (18,19) and rat gastric G-cells (17). We recently demonstrated that CART is expressed in several islet cell types, including β-cells, during rat development, and in pancreatic neurons innervating the islets (18). We have also shown that CART-deficient mice have impaired glucose-stimulated insulin secretion (GSIS) and impaired glucose tolerance explained by islet dysfunction (20).

Now, we have examined the role of CART 55–102 as a regulator of islet hormone secretion using INS-1(832/13) cells (21) and isolated rat islets. Further, CART localization and expression in two different rat models of type 2 diabetes, dexamethasone (DEX)-treated rats (22,23, rev. in 24) and Goto-Kakizaki (GK) rats (25), were examined.

RESEARCH DESIGN AND METHODS

All rats were from M&B (Ry, Denmark). For secretion studies, female Sprague Dawley (SD) rats (200 g) were used. Female (225 g, $n = 14$) and male (330 g, $n = 14$) SD rats were injected once daily with DEX, 2 mg/kg i.p. (Sigma-Aldrich, St Louis, MO) for 12 days. Female ($n = 6$) and male ($n = 6$) control rats were given an equal volume of saline once daily for 12 days (26,27). On day 12, six female and six male DEX-treated rats and all control rats were killed. The remaining animals were left to recover and killed after 5 ($n = 4$) or 10 ($n = 4$) days. Blood samples obtained by heart puncture at sacrifice were centrifuged and stored at −80°C for further handling. In addition, 6-month-old GK rats ($n = 6$) and Wistar rats (the parent strain of GK rats) ($n = 6$) of both sexes were used. Specimens for histochemical analysis were dissected and processed as previously described (18). The experiments were approved by the Regional Animal Ethics Committee in Lund, Sweden.

CART peptide. CART 55–102, rat long form (American Peptide, Sunnyvale, CA) was used. It was shown to be active since it increased locomotor activity after injection into the ventral tegmental area of the brain (28).

Insulin secretion assay on INS-1(832/13) cells and radioimmunoassay. INS-1(832/13) cells were seeded in 24-well plates and grown to confluence (21). Cells were preincubated in HEPES-buffered saline solution (HBSS): 114 mmol/l NaCl, 4.7 mmol/l KCl, 1.2 mmol/l KH_2PO_4, 1.16 mmol/l $MgSO_4$, 20 mmol/l HEPES, 2.5 mmol/l $CaCl_2$, 25.5 mmol/l $NaHCO_3$, 0.2% BSA, pH 7.2, with 3 mmol/l glucose for 2 h. For assay of insulin secretion, cells were incubated for 1 h in HBSS with 3 mmol/l glucose or 15 mmol/l glucose with 1–100 nmol/l of CART 55–102. In addition, 2.5 μmol/l forskolin (Sigma-Aldrich), a stimulator of adenylate cyclase activity, 100 μmol/l isobutyl-methylxanthine (IBMX) (Sigma-Aldrich), an inhibitor of phosphodiesterase activity, and 100 nmol/l glucagon-like peptide-1 (GLP-1) (Sigma-Aldrich), an incretin hormone known to stimulate β-cell adenylate cyclase activity, were used as insulin secretagogues. Insulin released into the buffer during the 1-h static incubation was determined by radioimmunoassay (Coat-a-count; DPC, Los Angeles, CA).

Secretion from isolated islets and radioimmunoassay for insulin, glucagon and somatostatin. Rat islets were isolated by standard collagenase digestion and handpicked under a stereomicroscope. Three rats were used for each experiment. Before experiments, islets were kept in HBSS containing 2.8 mmol/l glucose for 1 h at 37°C. Then, three islets for each condition were transferred to a 96-well plate kept on ice and containing 200 μl HBSS per well, with or without 1–100 nmol/l CART 55–102, and with or without 100 nmol/l GLP-1 at 2.8 or 16.7 mmol/l glucose. In addition, the protein kinase A (PKA) inhibitor H89 (0.5 μmol/l) (Biomol Research Labs, Plymouth Meeting, PA) and carbachol (100 μmol/l) (Sigma-Aldrich), a cholinergic agonist, were used as described in RESULTS. Following transfer of islets, the plate was incubated at

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CART, cocaine- and amphetamine-regulated transcript; DEX, dexamethasone; GLP-1, glucagon-like peptide-1; GSIS, glucose-stimulated insulin secretion; HBSS, HEPES-buffered saline solution; IBMX, isobutyl-methylxanthine; PKA, protein kinase A; PP, pancreatic polypeptide; TEM, transmission electron microscope.

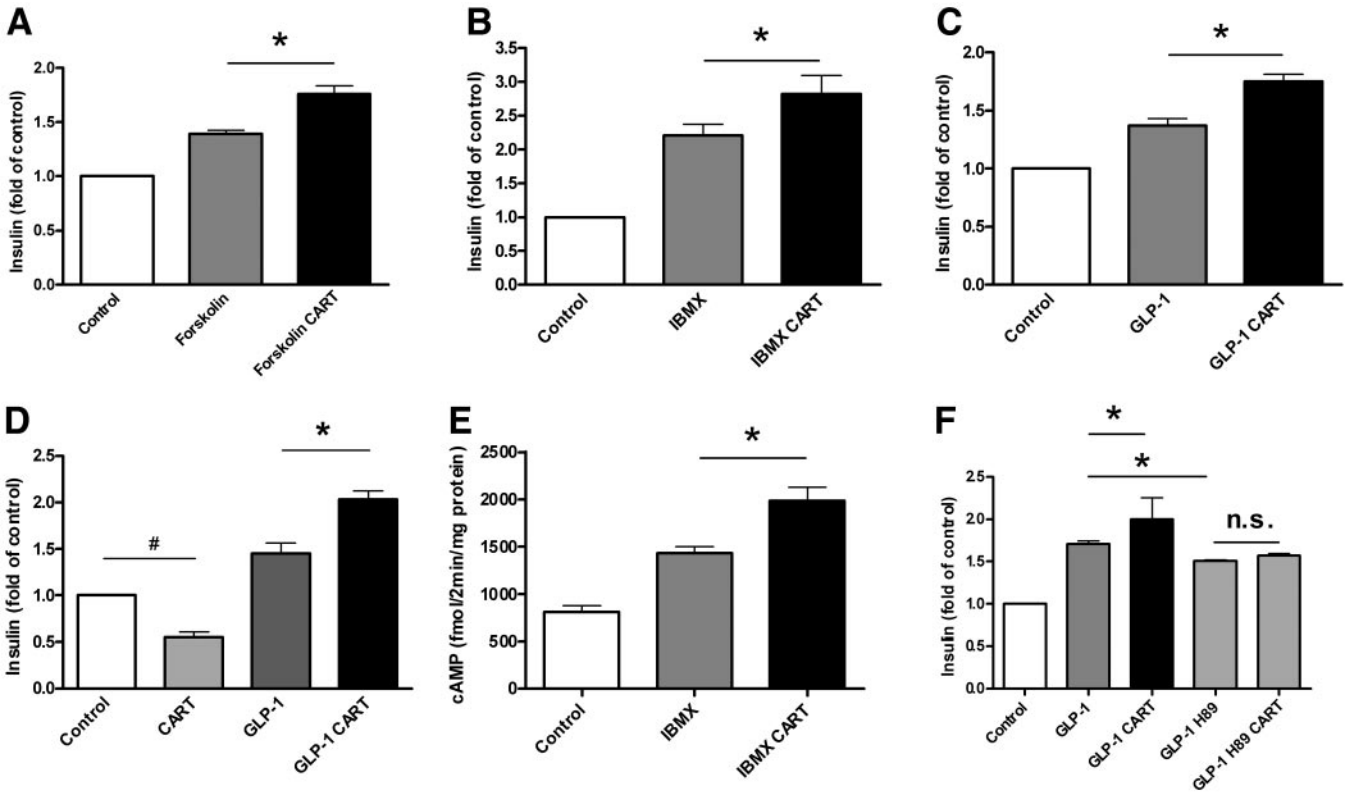

FIG. 1. *A–C*: Effect of CART (100 nmol/l) on insulin secretion from INS-1(832/13) cells stimulated with 15 mmol/l glucose (Control) or 15 mmol/l glucose in combination with forskolin (*A*), IBMX (*B*), or GLP-1 (*C*). CART augments forskolin, IBMX, and GLP-1–stimulated insulin secretion. *$P < 0.01$. *D*: Effect of CART on insulin secretion from SD rat islets stimulated with 16.7 mmol/l glucose (Control) or 16.7 mmol/l glucose and GLP-1. CART augments GLP-1–stimulated insulin secretion but inhibits insulin secretion stimulated with glucose alone. *$P < 0.01$; #$P < 0.05$. *E*: Effect of CART on intracellular cAMP content in INS-1(832/13) cells stimulated with 15 mmol/l glucose alone (Control) or with IBMX. CART increases intracellular cAMP content. *$P < 0.05$. *F*: Effect of CART on insulin secretion from rat islets stimulated with 16.7 mmol/l glucose alone (Control) or with GLP-1 and H89. The effect of CART on GLP-1–mediated insulin secretion from rat islets was abolished with addition of the PKA-inhibitor H89. *$P < 0.01$; n.s. ($P > 0.05$). Islet data from four different experiments with eight separate islet incubations for each condition; INS-1 cell data from four different experiments run in quadruplicate.

37°C for 1 h. Thereafter, a sample from the buffer was removed for radiochemical measurements of insulin (Linco Research, St. Louis, MO), glucagon (Linco Research), and somatostatin (EuroDiagnostica, Malmö, Sweden).

cAMP measurement on INS-1(832/13) cells. Cells were grown to confluence in 12-well plates (21) and preincubated in HBSS as above. The medium was changed to HBSS containing 15 mmol/l glucose and 100 μmol/l IBMX, or 100 nmol/l GLP-1 with or without 100 nmol/l CART, and the cells were incubated for 2 min. The reactions were terminated by addition of ice-cold ethanol reaching a final concentration of 65%. Cells were scraped off and the extracts were centrifuged at 2000*g* at 4° for 15 min, transferred to new tubes, and evaporated. cAMP was measured by radioimmunoassay (Amersham, Uppsala, Sweden).

Plasma glucose analysis. Glucose was measured using the glucose oxidase method (Thermo Trace, Victoria, Australia).

Immunocytochemistry. Indirect immunofluorescence was used throughout and performed as desribed (18). The following primary antibodies were used: CART, code 12/D, dilution 1:1,280 (Cocalico, Reamstown, PA); somatostatin, code V1169, dilution 1:400 (Biomeda, Foster City, CA); proinsulin, code 9,003, dilution 1:2,560 (EuroDiagnostica), glucagon, code 8708, dilution 1:5,120 (EuroDiagnostica); and pancreatic polypeptide, code AHP 515, dilution 1:640 (Serotec, Oxford, U.K.). The specificity of immunostaining was tested by using primary antisera preabsorbed with homologus antigen (100 μg of peptide per milliliter antiserum in working dilution) or by omission of primary antibodies.

Electron microscopy and immunogold. Specimens were fixed and processed as previously described (29) and thereafter embedded in Lowicryl HM20 (TAAB, Reading, U.K.). Ultrathin sections were cut and placed on gold grids. Immunogold was performed as previously described (29) with CART antiserum (diluted 1:100) and protein A-gold (10 nm diameter) solution (diluted 1:20; Amersham). Grids were routinely contrasted (29) and examined in a Philips CM10 transmission electron microscope (TEM).

In situ hybridization. In situ hybridization was carried out as previously described (18). A mix of two synthetic 30 mer oligodeoxyribonucleotide probes (18) for CART mRNA, 3′-endtailed by [S^{35}]dATP (NEN, Stockholm, Sweden) (5) was used. For control purposes, hybridization in the presence of 100-fold excess of unlabeled probe was performed.

Image analysis and morphometry. Immunofluorescence was examined in an epifluorescence microscope. By changing filters the location of the different secondary antibodies in triple staining was determined. Images were captured with a digital camera. Islets (n = 5–8 per animal) were randomly selected from different parts of the sections. To quantify cells coexpressing CART and islet hormones (insulin and somatostatin), immunoreactive cells were counted, and the mean percentage of each of the various islet cell types expressing CART was calculated, as previously described (18). In situ hybridization radiolabeling was examined in dark or bright field and images were captured with a digital camera. National Institutes of Health image software was used to quantify the density of labeling for CART mRNA, as previously described (18,30). For this, islets were selected as above.

Statistical analysis. Results are shown as means ± SE. Secretion data were analyzed by a one-way ANOVA followed by Bonferroni's test post hoc or by Student's paired t test. Morphometrical data were analyzed by Student's unpaired t test. Differences with a value of $P < 0.05$ were considered significant.

RESULTS

Secretion studies

Effects of CART on insulin secretion from clonal β-cells. INS-1(832/13) cells were incubated at 3 or 15 mmol/l glucose with increasing concentrations of CART. Raising the glucose in the medium from 3 to 15 mmol/l provoked a fivefold increase in insulin secretion during the 1 h static incubation. CART (1–100 nmol/l) had no effect on insulin secretion under these conditions (data not shown). However, CART potentiated cAMP-enhanced GSIS. Thus,

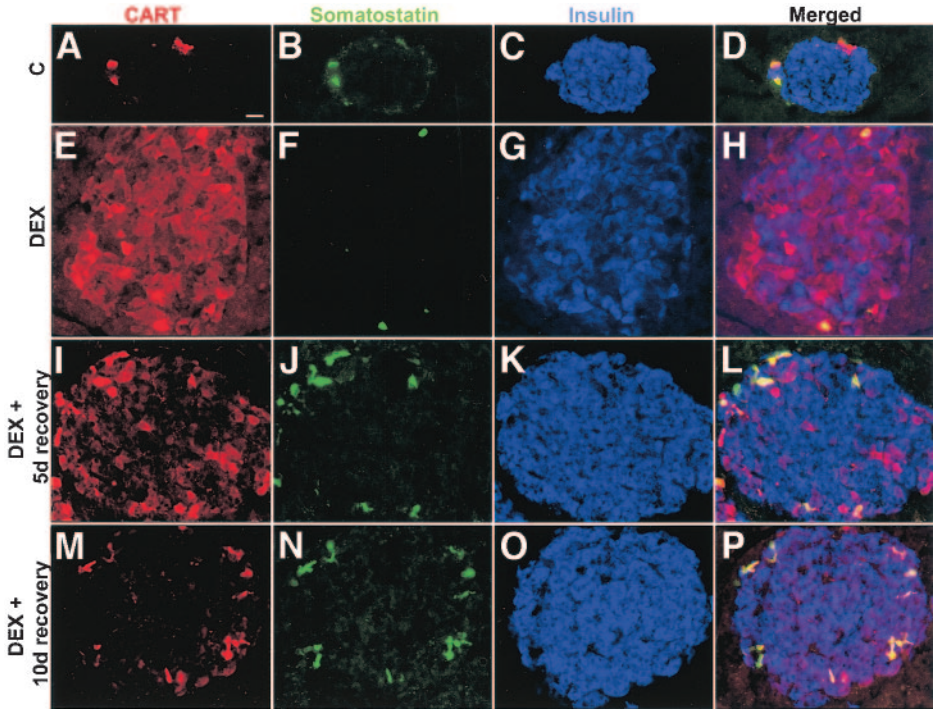

FIG. 2. Islets triple immunostained for CART, somatostatin, and insulin. In controls, CART is mainly expressed in δ-cells (*A–D*). After DEX treatment, CART is robustly upregulated in the β-cells (*E–H*). After 5 days of recovery, CART expression in β-cells is reduced (*I–L*) but still higher than in controls. After 10 days of recovery, the pattern of CART expression is indistinguishable from controls (*M–P*). Bar = 20 μm.

at 15 mmol/l glucose, CART (100 nmol/l) evoked a 25 ± 5% ($P < 0.01$) augmentation of forskolin-stimulated GSIS (Fig. 1*A*), a 25 ± 5% ($P < 0.001$) augmentation of IBMX-stimulated GSIS (Fig. 1*B*), and a 30 ± 2% ($P < 0.0001$) increase of GLP-1-stimulated GSIS (Fig. 1*C*). Dose-response experiments revealed that CART at the concentrations tested was maximally effective at 100 nmol/l (data not shown).

Effects of CART on islet hormone secretion from isolated islets. SD rat islets were incubated at 2.8 or 16.7 mmol/l glucose. The rise in glucose provoked a 15-fold increase in insulin release during the 1-h static incubation. At 16.7 mmol/l glucose, CART (100 nmol/l) reduced insulin secretion to 55 ± 8% ($P < 0.05$) of that in control islets (Fig. 1*D*). At 16.7 mmol/l glucose, CART also reduced secretion of glucagon to 74 ± 13% ($P < 0.01$) of control and secretion of somatostatin to 60 ± 15% ($P < 0.01$) of control. At 2.8 mmol/l glucose there was no effect of CART on insulin secretion (data not shown). On the other hand, CART potentiated cAMP-enhanced GSIS. Thus, CART caused a 42 ± 7% ($P < 0.01$) raise of GLP-1–stimulated GSIS (Fig. 1*D*).

Effects of CART on cAMP and PKA. To find a mechanism for the effect of CART on cAMP-enhanced GSIS, we first measured cAMP in INS-1(832/13) cells. The cAMP levels in cells treated with IBMX together with CART were 40 ± 20% higher than in cells treated with IBMX alone ($P < 0.05$) (Fig. 1*E*). cAMP levels in cells treated with GLP-1 and CART were 30 ± 8% higher (four experiments run in quadruplicate; $P < 0.01$) than in cells treated with GLP-1 alone, indicating an additive effect of the peptides.

To explore whether PKA-dependent or -independent mechanisms account for the observed effects, we used the PKA inhibitor H89. H89 completely abolished the potentiating effect of CART on GLP-1-stimulated GSIS (Fig. 1*F*). This strongly suggests that the potentiating effect of CART on cAMP-enhanced GSIS is mediated via PKA. Screening for alternative mechanisms of the effect of CART on insulin secretion revealed that CART had no effect on that mediated by carbachol (data not shown).

CART in rat type 2 diabetes models. Since we found CART capable of regulating islet hormone secretion, we examined CART expression in two different type 2 diabetes models.

DEX-treated rats

Immunocytochemistry. Triple immunostainings for CART/somatostatin/insulin and CART/pancreatic polypeptide/glucagon showed that in control rats CART immunoreactivity was largely restricted to the δ-cells and pancreatic neurons (Fig. 2*A–D*); however, a minor subpopulation of the β-cells displayed weak CART immunoreactivity (data not shown). In DEX-treated rats, in addition to δ-cells and neurons, a great proportion of the β-cells was CART immunoreactive (Fig. 2*E–H*); there were no CART-immunoreactive PP-cells or α-cells. Quantification revealed a 10-fold increase in the relative number of β-cells coexpressing CART in DEX-treated rats compared with controls (40 ± 2 vs. 3.5 ± 1%; $P < 0.001$). No differences in relative number of CART-immunoreactive δ-cells could be detected between DEX-treated rats and controls (69 ± 8 vs. 85 ± 5%; NS). In addition, neuronal CART immunoreactivity was more intense in DEX-treated rats than in controls (data not shown). Five days after termination of DEX treatment (Fig. 2*I–L*), the number of CART-immunoreactive β-cells was reduced by 25% ($P < 0.02$) compared with the maximum but was still eight times ($P < 0.001$) higher than in the controls. Ten days after termination (Fig. 2*M–P*), CART immunoreactivity was reduced to a level comparable with the controls ($P > 0.1$ vs. control) (Fig. 2). Quantification results are summarized in Fig. 5*A*. In addition, examination of CART expression in the G-cells in the gastric antrum, recently shown to express CART (17), did not reveal any difference between DEX-treated rats and control rats (data not shown).

Immunogold and TEM. To localize CART in β-cells at the subcellular level, immunogold labeling for CART was performed. TEM examination revealed that labeling for CART was present in the secretory granules of β-cells (Fig. 3*A*) and δ-cells (Fig. 3*C*). Notably, in the β-cells the gold particles were mainly localized to the electron-lucent

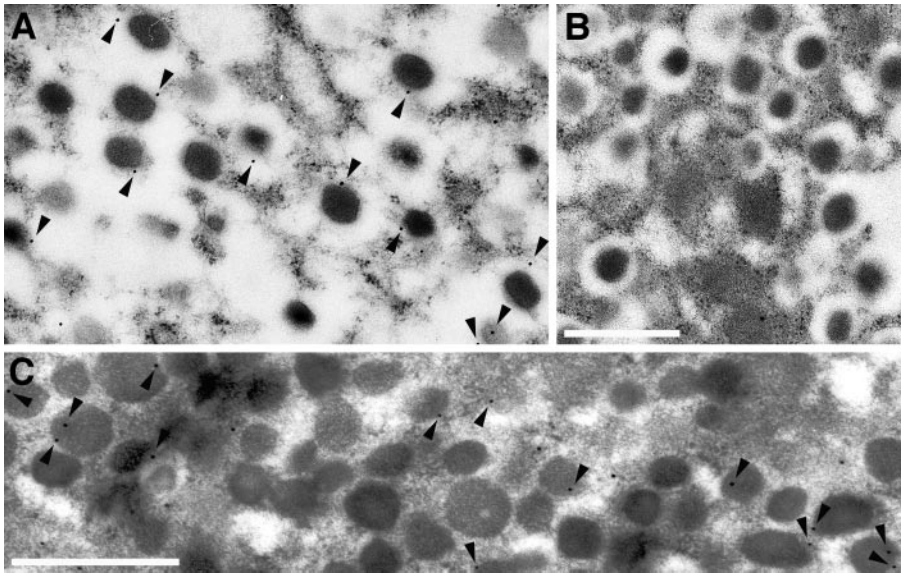

FIG. 3. Electron micrographs of β-cells (*A* and *B*) and δ-cell (*C*) from DEX-treated rat. *A* and *C*: immunogold labeling for CART. Arrowheads indicate CART labeling. Note that CART labeling is localized to the halos of the β-cell granules (*A*) and not in the dense cores. *B*: Negative control displaying no CART labeling. Bars = 0.5 nm; in *B* for *A and B*; in *C* for *C*.

halos of the granules, between the dense core and the limiting membrane. Control grids displayed only very weak scattered unspecific labeling (Fig. 3*B*).

In situ hybridization. In control rats, weak-to-moderate labeling for CART mRNA was restricted to single cells at the islet periphery (Fig. 4*A*). In DEX-treated rats, dense labeling for CART mRNA was seen also over the central area of the islets (Fig. 4*B*). Quantification of density of labeling for CART mRNA revealed 10- ± 1-fold higher expression in DEX-treated rats than in controls ($P < 0.001$). After 5 days of recovery CART mRNA expression was markedly reduced, but still 2 ± 0.2-fold higher than in controls ($P < 0.02$) (Fig. 4*C*). After 10 days of recovery, the level of CART mRNA expression was indistinguishable from that of the controls (Fig. 4*D*). The results of mRNA density quantification are summarized in Fig. 5*B*.

Body weight development and plasma glucose. DEX-treated rats lost weight during the treatment period and regained weight after recovery as previously described (3). Further, the DEX-treated rats had almost twofold higher plasma glucose than the controls (380 + 45 and 207 + 13 mg/dl; $P < 0.01$). Five and 10 days after recovery, the glucose levels were indistinguishable from control (200 ± 10 and 208 ± 8 mg/dl).

GK rats

Immunocytochemistry. In Wistar control rats, CART was mainly located to the δ-cells (Fig. 6*E–H*). In GK rats, a great proportion of the β-cells was CART immunoreactive (Fig. 6*A–D*) and quantification revealed that 60 ± 7% of the β-cells contained CART immunoreactivity compared with 2 ± 1% in the controls ($P < 0.001$). There were no differences in relative number of CART-immunoreactive δ-cells (73 ± 4 vs. 85 ± 6%; NS) or no obvious difference in neuronal CART immunoreactivity (data not shown). There were no CART-immunoreactive PP-cells or α-cells in GK rats or controls.

In situ hybridization. In Wistar control rats, moderate labeling for CART mRNA was seen in single cells at the islet periphery. In GK rats, dense labeling for CART mRNA was seen also over the central parts of the islets (data not shown).

Insulin secretion. We next studied whether CART affects insulin secretion in islets of GK rats. Islets from GK and Wistar control rats were incubated with 2.8 or 16.7 mmol/l glucose for 1 h as described above. GSIS was blunted in GK islets (460 ± 74–1,485 ± 200 pg insulin · islet^{-1} · h^{-1}) compared with controls (441 ± 80–2,792 ± 200 pg insulin · islet^{-1} · h^{-1}) (3- ± 0.2- vs. 6- ± 1-fold increase; $P < 0.02$). Addition of GLP-1 caused a 40 ± 2% increase of GSIS from control islets and 30 ± 1% increase of GSIS from GK islets. Addition of CART caused a further stimulation in both groups. Interestingly, the potentiating effect of CART on GLP-1-stimulated GSIS was higher in GK islets than in control islets (58 ± 14 vs. 15 ± 6%; $P < 0.05$). On the other hand, in the absence of GLP-1, CART caused similar inhibition of GSIS in both groups (GK 74 ± 9 vs. Wistar 67 ± 9% of control, respectively; NS). The results are summarized in Fig. 7.

DISCUSSION

We recently demonstrated that CART is expressed in several islet cell types during rat development (18) and that CART deficient mice display impaired islet function (20). Here we show that CART regulates hormone secretion from clonal β-cells and rat islets and that CART is upregulated in the β-cells of two mechanistically different rat models of type 2 diabetes.

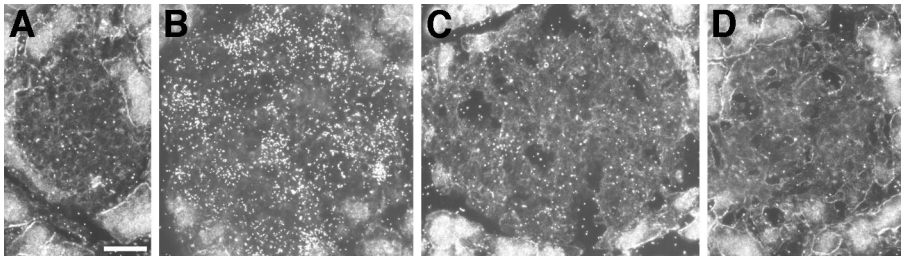

FIG. 4. In situ hybridization (dark field) for CART mRNA. In controls, weak CART expression is seen at the islet periphery (*A*). After DEX treatment, CART expression is robustly upregulated over central parts of the islets (*B*). After 5 days of recovery labeling is markedly reduced (*C*), and after 10 days there was no difference to controls (*D*). Bar = 50 μm.

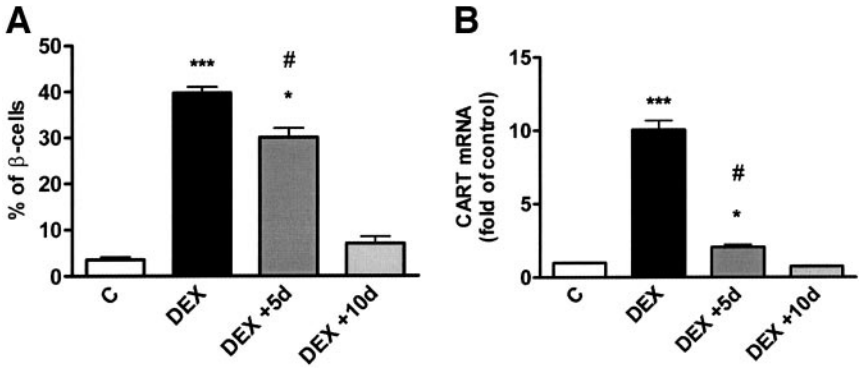

FIG. 5. CART-expressing β-cells (*left*) and CART mRNA (*right*) after DEX treatment and 5 (5d) and 10 (10d) days of recovery. ***P < 0.01 DEX vs. control (C); *P < 0.05 5d vs. C; #P < 0.05 5d vs. DEX.

A main finding is that CART affected islet hormone secretion. Thus, in both clonal β-cells and isolated rat islets, CART potentiated GSIS amplified through adenylate cyclase activation by forskolin or GLP-1. The augmentation was paralleled by an increase in intracellular cAMP and was abolished after PKA-inhibition with H89. Thus, the potentiating effect of CART on GLP-1–mediated GSIS could be explained by an amplified rise in cAMP and possibly a stronger ensuing activation of the PKA-dependent pathway. Further, CART raised GSIS amplified through blockade of phosphodiesterase with IBMX. The latter suggests that CART does not exert its effect by inhibiting phosphodiesterase activity. In the present study we were unable to find any effect of CART on GSIS from clonal β-cells in the absence of cAMP-elevating agents. This is in accordance with a previous report on INS-1E cells (31) and indicates that a state of high cAMP is needed for CART to exert the potentiating effect. Although the CART receptor is still not identified one may speculate that the state of high cAMP levels (caused by forskolin, IBMX, or GLP-1) induces activation, e.g., phosphorylation, of the CART receptor to make it susceptible for its ligand. Our data that CART itself causes a further increase in intracellular cAMP under already elevated conditions (as caused by the high concentration of IBMX or by GLP-1) indicates that the CART receptor may be coupled to an adenylate cyclase. Taken together our data suggest that at high cAMP levels CART acts to further increase cAMP. Thus, this report is to our knowledge the first to suggest a mechanism of action for CART in the β-cell. However, there may be alternative explanations for the CART effect, e.g., generation of an independent pool of cAMP. Recently, Lakatos et al. (32) presented data suggesting that CART activates the extracellular signal-regulated kinase pathway via a putative G-protein coupled receptor in a pituitary-derived cell line. These data are intriguing and may in the near future provide explanations for many CART effects. Together with our present data, this indicates that multiple CART receptor subtypes may exist.

In the absence of cAMP elevators, CART decreased insulin-, glucagon-, and somatostatin secretion. This is partly in agreement with Wang et al. (33), who presented data indicating inhibitory effects of CART on GSIS from isolated islets. This suggests that at basal cAMP levels, CART acts via an alternative mechanism and that CART may under these conditions act as a general inhibitor of islet hormone secretion. The inhibitory effect of CART on insulin secretion could be indirect and due to the inhibited release of glucagon, indeed a known stimulator of insulin secretion via an increase in cAMP. If so, this may also indicate that there exists more than one type of CART receptor and that the CART receptor on the α-cells can be activated by CART without a previous activation as may be caused by cAMP in the β-cells. The possibility of an indirect effect gains support from our finding that CART did not inhibit basal or glucose stimulated insulin secretion from INS-1(832/13) cells, since there is no source of glucagon in that model system (34).

We recently showed that CART-deficient mice have impaired GSIS and impaired glucose elimination in vivo, and that the absence of CART caused impaired insulin secretion from isolated islets (20). Interestingly, humans with a missense mutation in the CART gene were reported to have an elevated incidence of type 2 diabetes (35). Together with the present finding that CART is a regulator of islet hormone secretion, the data favor a role for CART in the control of glucose homeostasis. Therefore, a role for CART in type 2 diabetes pathophysiology cannot be ruled out.

In view of our present data on CART and islet hormone secretion, our finding of a robust upregulation of CART in the β-cells of two mechanistically different models of type 2 diabetes is intriguing. The GK rat is a well-established polygenic model of inherited type 2 diabetes (25). In vivo administration of glucocorticoids (DEX) is a commonly used experimental model for inducing insulin resistance with glucose intolerance and hyperinsulinemia and a compensatory hypertrophy and hyperplasia of the β-cells

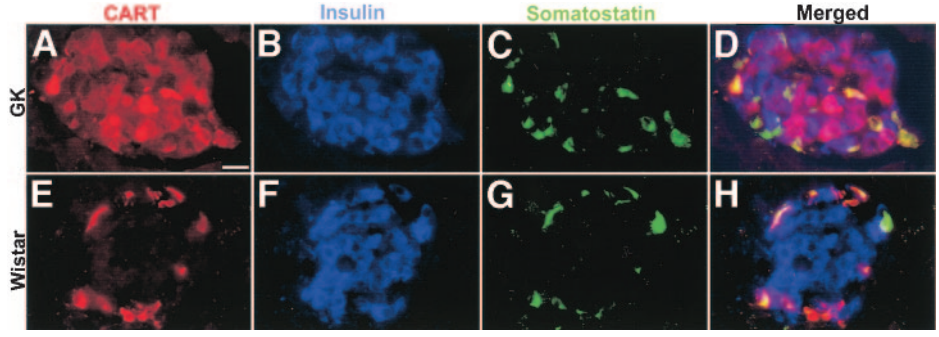

FIG. 6. Islets triple immunostained for CART, insulin, and somatostatin. In Wistar rats (*bottom*), CART is expressed in δ-cells. In GK rats (*top*), CART is robustly upregulated in the β-cells. Bar = 20 μm.

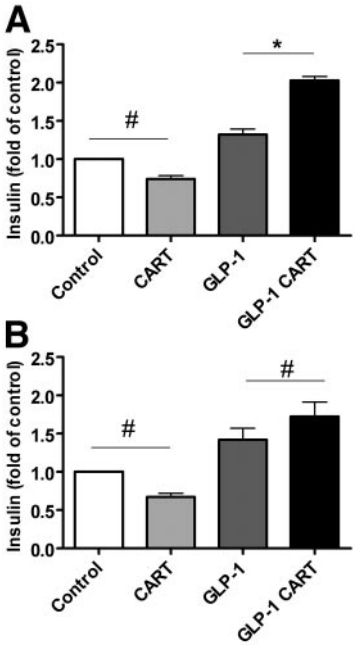

FIG. 7. Effect of CART (100 nmol/l) on insulin secretion from isolated rat islets stimulated with 16.7 mmol/l glucose (Control) or 16.7 mmol/l glucose in combination with GLP-1. CART cause a greater increase in GLP-1–mediated insulin secretion in GK rats (*A*) than in Wistar rats (*B*). There was no difference in the absence of GLP-1. *$P < 0.01$, #$P < 0.05$. Data are from four different experiments with eight separate islet incubations for each condition.

(22,23, rev. in 24). The upregulation of islet CART after DEX treatment could be interpreted either as a direct effect of high glucocorticoids per se or as secondary to the type 2 diabetes phenotype. Balkan et al. (36) and Vrang et al. (37) reported that hypothalamic CART is regulated by glucocorticoids, and Vicentic et al. (38) found that glucocorticoids affect CART levels in blood. Although Vrang et al. (37) noted the presence of a glucocorticoid response element upstream (6.2 kb) of the CART proximal promoter, and Balkan et al. and others (36,39) point out that glucocorticoid receptors can interact with transcription factors at certain sites in the CART proximal promoter, it is not yet known whether these sites are functional in the regulation of CART. Thus, the mechanism underlying the effect of glucocorticoids on CART remains unclear. The present finding of upregulation of CART also in GK rat β-cells, together with our recent data on CART upregulation in β-cells of Zucker diabetic fatty rats (40) and ob/ob mice (N.W., D.X. Gram, F.S., unpublished data), suggest that islet CART is not necessarily regulated by high glucocorticoids. Rather, the increase could be secondary to the insulin resistance and the metabolic perturbations with ensuing increase in the β-cell burden to compensate for the increased insulin demand and β-cell growth. It is of interest in this context that in the neonatal rat, when islet growth is fast, islet CART is markedly upregulated (18). Our present findings of lacking upregulation of CART in islet δ-cells and gastric G-cells, known to express CART (17), support the possibility that the islet CART upregulation is secondary to the type 2 diabetes phenotype and not a general effect on CART transcription.

Both GK rats and Wistar control rats responded to CART in a similar fashion, as did the SD rats. Interestingly, although there was no difference in the response to GLP-1, the potentiating effect of CART on GLP-1–mediated GSIS was significantly higher in GK rats than in Wistar rats. A possible explanation for this may be that the upregulated endogenous CART in the β-cells is coupled to a higher sensitivity of the cells to exogenous CART. The mechanism for the increased sensitivity to CART has yet to be elucidated.

The data presented here are all from experiments employing a concentration of CART of 100 nmol/l. The circulating concentrations of CART in plasma are in the range 10–150 pmol/l (19, 38, 41). Although our concentration is rather high in terms of circulating CART, it is reasonable to assume that CART released in an autocrine/paracrine fashion may reach this level in the vicinity of β-cells. In addition, all data are from experiments with CART 55–102. Although CART 62–102 has been shown to have qualitatively similar effects as CART 55–102 (42–44), the effects of other forms of CART on islet hormone secretion remain to be elucidated.

The upregulation of CART in DEX-treated rats was reversible and characterized by a rapid decrease in CART mRNA expression and peptide. This indicates a high plasticity of CART expression in the islets. Further, the decrease in CART mRNA coincides in time with the normalization of plasma glucose. The high plasticity of CART expression is reminiscent of the reaction of neuropeptide Y in rat islet β-cells after DEX treatment (3). Further, it should be emphasized that there are multiple sources of islet CART (δ-cells, β-cells, and neurons), suggesting multiple functions and adding to the complexity of CART function.

Our present ultrastructural finding of CART insulin resistance in β-cell granules indicates that CART may be cosecreted with insulin; however, the amount secreted from three SD islets were below the detection limit of the radioimmunoassay (N.W., M.B., H.M., F.S., unpublished observations). The localization of CART to the halos, outside the dense core, of the granules agrees with that of certain other β-cell peptides such as islet amyloid polypeptide (45), while insulin resides in the dense core (46). The reason for this compartmentalisation has yet to be uncovered.

In conclusion, our study provides further evidence of a role for CART as a regulator of islet hormone secretion. In addition, we show for the first time that CART is induced in the β-cells under conditions of β-cell challenge. In view of successful clinical trials with GLP-1 (47,48), the potential of CART or CART analogs in combination with GLP-1 in the treatment of type 2 diabetes remains to be evaluated.

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