

CART decreases islet blood flow, but has no effect on total pancreatic blood flow and glucose tolerance in anesthetized rats

Carl Johan Drott^{a,*}, Daniel Norman^a, Daniel Espes^{a,b}

^a Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden

^b Department of Medical Sciences, Uppsala University, Uppsala, Sweden

ARTICLE INFO

Keywords:

CART
Pancreatic islets
Blood flow
Vascular

ABSTRACT

Cocaine- and amphetamine-regulated transcript (CART) is a neurotransmitter and hormone, involved in the regulation of e.g. food intake, body weight, reward and addiction, and stress response. CART has also been found to affect insulin secretion and beta cell morphology, both in vivo and in vitro. Furthermore, CART affects regulation of the cardiovascular system and helps to modulate vascular tone. The present study evaluated the local effect of CART on the pancreatic and islet circulation and function. CART (25 µg/h) or saline, combinations of CART and endothelin-A receptor antagonist (BQ123; 100 µg/kg), and glucose (2 g/kg) were intravenously infused in Sprague Dawley rats followed by blood flow measurements using a microsphere technique. Separately, CART-infused animals underwent an intravenous glucose tolerance test (ivGTT). The direct effect of CART on insulin release was investigated using isolated islets from Sprague Dawley rats. CART reduced islet blood flow, without reduction in total pancreatic blood flow. The normal glucose-induced islet blood flow increase was diminished by CART, albeit still present. Simultaneously, CART had no effect on systemic-, intestinal- or renal blood flow. The endothelin-A receptor antagonist BQ123 together with CART had no pancreatic vascular effects. We found that CART has pronounced vascular constrictive actions restricted to the pancreatic islet circulation but had no effect on insulin release neither in vivo nor in vitro. The mechanisms behind the vascular effects are still unknown, but may reflect a direct action on pancreatic blood vessels.

1. Introduction

Cocaine and amphetamine regulated transcript (CART) is a peptide transcribed from the *CARTPT* gene, which is widely expressed in the central- and peripheral nervous system, as well as in the endocrine cells in the pituitary [1], adrenal medulla [1], antral gastrin producing G-cells in the stomach [2], and in pancreatic islets and neural tissue of the endocrine pancreas [3]. CART was initially identified as an mRNA transcript

linked to acute psychostimulant use, reacting on either cocaine or amphetamine [4]. Further studies have found a role for CART as a key neurotransmitter and anorexigenic hormone [5,6]. CART is involved in the regulation of many diverse biological processes such as feeding

behavior, regulation of food intake [7], maintenance of body weight, reward and addiction [7], stress response, and in mediating the locomotor effects of psychostimulants [6].

In the pancreas, CART is expressed both by islet endocrine cells and by parasympathetic and sensory nerves innervating the islets. This suggests that CART may interact in the parasympathetic control of islet function, in the regulation of insulin secretion and in the stimulation of pancreatic exocrine secretion [3,8,9]. The expression of CART is species dependent [3,9–11], but has been found to be upregulated during fetal and neonatal development in rodents [8,12]. In adult rats CART is expressed in delta cells and in a minor subpopulation of beta cells [11], whereas in adult mice CART is mainly expressed in nerve fibers [8], and in a subpopulation of beta cells. In humans CART has been found to be

Abbreviations: ANOVA, Analysis of variance; BQ-123, Selective ET_A endothelin receptor antagonist; cAMP, cyclic adenosine monophosphate; CART, Cocaine and amphetamine regulated transcript; CNS, Central nervous system; ELISA, Enzyme Linked ImmunoSorbent Assay; ET-1, Endothelin-1; ET_A, Endothelin-1 receptor type A; ET_B, Endothelin-1 receptor type B; GLP-1, Glucagon-like peptide-1; GSIS, Glucose stimulated insulin secretion; HEPES, Hydroxyethyl-piperazine-ethanesulfonic acid; ivGT, Intravenous Glucose Tolerance Test; IBF, Islet Blood Flow; K₂ EDTA, Di-Potassium Ethylenediaminetetraacetic acid; KO, Knock Out; KRBH Krebs, Ringer Bicarbonate Buffer; NO, Nitric Oxide; PBF, Pancreatic Blood Flow; PK, A protein kinase A; SEM, Standard error of the mean.

* Corresponding author at: Department of Medical Cell Biology, Uppsala University, Husargatan 3, Box 571, SE-751 23 Uppsala, Sweden.

E-mail address: carljohan.drott@mcb.uu.se (C.J. Drott).

<https://doi.org/10.1016/j.peptides.2020.170431>

Received 24 March 2020; Received in revised form 12 October 2020; Accepted 12 October 2020

Available online 21 October 2020

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expressed in both alpha- and beta cells [12]. The levels of CART have also been found to be upregulated in several experimental models of type 2 diabetes as well as in pancreas from human type 2 diabetic patients [8,11,12]. Experimental studies have found that CART can promote glucose-stimulated insulin secretion in beta cells in rats via cAMP/PKA-dependent pathways [6,11]. Furthermore, it has been demonstrated that the insulin secretion is decreased in CART knock-out (KO) mice, both in vivo and in vitro [13], and that these mice become obese [14]. The effect on insulin secretion has been observed also in young CART KO mice, suggesting that CART impacts normal beta cell growth and function [8,13]. In humans, different alterations resulting in CART surplus have been associated with reduced metabolic rate, hyperphagia, obesity and elevated incidence of type 2 diabetes [15–17] indicating a role for CART in regulation of body weight and metabolic control [16].

CART stimulates insulin secretion in both mouse and human islets, but the effect in rat has varied between studies [9]. In rats, if CART is administered in combination with a cAMP-elevating agent, it improves insulin secretion [11]. CART does also affect the vasculature and has been found to be localized to the nucleus of the solitary tract and the area postrema, and to play a role in the central nervous regulation of the cardiovascular system [18]. CART is released in hypothalamic-pituitary portal circulation in response to nitric oxide (NO) induced hypotension [19], suggesting that CART has a direct effect on the regulation of the vascular tonus [20]. CART has previously been shown to exert vasoconstricting effects on arterioles of the central nervous system (CNS), and the effect is suggested to involve the ET_A receptor in the endothelin system [20,21]. Endothelin-1 (ET-1) is produced in the endothelium and is present in the pancreatic vasculature and predominantly in the islet cells [22]. ET-1 has been found to reduce basal pancreatic blood flow (PBF) and especially islet blood flow (IBF) through the ET-A receptor, and has therefore been suggested as an important regulator of PBF and IBF [23].

Despite many studies in the field, the receptor for CART has still not been identified [24–26]. Similarly, despite the proposed actions of CART on islet physiology and implications in type 2 diabetes, the effect of CART on PBF and IBF regulation has not been studied. This study tested the hypothesis that CART might act as a regulator of the pancreatic vasculature and blood flow, and if so whether this effect was mediated via the endothelin system. In addition, the direct effect of CART on insulin secretion was investigated both in vivo and in vitro.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (M&B, Ry, Denmark) 12–16 weeks of age and weighing approximately 250–350 grams were housed with free access to pellet food and water. All protocols and all experiments were approved by the Animal Ethics Committee for Uppsala University, Uppsala, Sweden. The Guide for the care and use of laboratory animals, Eighth edition (2011) (<http://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf>) was followed, as well as specific national laws where applicable.

2.2. Blood flow measurements

Measurements of blood flow were performed with a microsphere technique as previously described in detail [27,28]. Briefly, animals (n = 7–8) were anesthetized using an intraperitoneal injection of thio-butabarbital sodium (120 mg/kg; SigmaAldrich Chemie GmbH, Steinheim, Germany) and placed on an operating table heated to 38.0 °C. The animals were tracheotomized, and polyethylene catheters were inserted into their ascending aorta through the right carotid artery, into their left femoral artery and into their left femoral vein. Blood samples for analysis of blood glucose concentration by test reagent strips (Freestyle Lite;

Abbott, Deerfield, IL, USA) and of serum insulin concentration (Rat insulin ELISA; Mercodia; Uppsala, Sweden) was collected from the tail vein and from the femoral artery, respectively. CART (25 µg/h, 1.0 mL/h; Tocris; Abingdon, UK) dissolved in saline, Glucose (2.0 g/kg, 1.0 mL/h, NovoNordisk; Bagsvaerd, Denmark), glucose together with CART, BQ123 (a selective ET_A-receptor antagonist, 100 µg/kg, 0.5 mL/h, or 1.0 mL/h, BW IV), BQ123 and CART dissolved in saline, or saline alone (1.0 mL/h) was infused for 30 or 60 min into the catheterised femoral vein (for details of groups see Table 1). The doses of CART and BQ-123 used in this study were based on previous studies [11, 20].

Approximately $1-2 \times 10^6$ non-radioactive black microspheres (E-Z Trac, IMT; Irvine, CA, USA) were injected via the right carotid artery into the ascending aorta. An arterial blood reference sample was collected for 60 s from the catheter in the femoral artery, starting immediately before the injection of microspheres. The exact arterial flow during this 60 s was confirmed in each experiment by weighing the sample. The pancreas, adrenal glands, duodenum, colon and kidneys were retrieved, dissected free from adipose tissue and weighed. All tissue samples were subjected to a freeze-thawing technique to visualize the microspheres and allow separate counting of intra-islet microspheres [27]. The microspheres present in the samples were counted in a microscope equipped with both dark and bright field illumination [27]. The reference blood collected during the experiment was transferred to glass microfiber filters with a pore size of <10 µm before the microspheres therein were counted. The organ blood flow was then calculated according to the formula: $Q_{org} = N_{org} \times Q_{ref} / N_{ref}$ where Q_{org} denotes organ blood flow (ml/min), Q_{ref} the flow of the reference sample (ml/min), N_{org} the number of microspheres present in the organ, and N_{ref} the number of microspheres present in each reference sample. In the adrenal glands, a difference in blood perfusion between the left and right gland of <20 % was used as measurement for equal distribution of the microspheres, otherwise the experiment was discarded.

2.3. Intravenous glucose tolerance test

Rats (n = 9) were anesthetized with pentobarbital sodium vet (60 mg/kg i.p.; APL, Sweden), and polyethylene catheters were placed in their left femoral artery and in their left femoral vein. CART (25 µg/h; 1.0 mL/h) dissolved in saline or saline alone (1.0 mL/h) was infused intravenously for 60 min. After 30 min of infusion of CART or saline, glucose was injected (300 mg/mL, 2.0 g/kg), i.e. the start of the ivGTT = minute 0. This design was chosen in order to study the acute effects of CART on glucose metabolism and insulin secretion in vivo.

Blood glucose was measured at -30, 0, 15, 30, 60 and 120 min after

Table 1
Infusion components in each group.

Control:	Infusion Ringer 1.0 mL/h for 60 min. At 57' a bolus dose of 1 mL Ringer is given. Microspheres at 60'
CART:	Infusion of CART (25 µg/h; 1.0 mL/h) dissolved in Ringer for 60 min. At 57' a bolus dose of 1 mL Ringer is given. Microspheres at 60'.
Glucose:	Infusion Ringer 1.0 mL/h for 60 min. At 57' a bolus dose of 1 mL 30% D-glucose is given. Microspheres at 60'.
Glucose + CART:	Infusion of CART (25 µg/h; 1.0 mL/h) dissolved in Ringer for 60 min. At 57' a bolus dose of 1 mL 30% D-glucose is given. Microspheres at 60'.
BQ 123 30 min:	Infusion Ringer 0.5 mL/h for 30 min. At 25' a bolus dose of BQ123 (100 µg/kg BW IV) Microspheres at 30'.
BQ 123 30 min + CART:	Infusion of CART (25 µg/h; 0.5 mL/h) dissolved in Ringer for 30 min. At 25' a bolus dose of BQ123 (100 µg/kg BW IV) Microspheres at 30'.
BQ 123 60 min:	Infusion Ringer 1.0 mL/h for 60 min. At 55' a bolus dose of BQ123 (100 µg/kg BW IV) Microspheres at 60'.
BQ 123 60 min + CART:	Infusion of CART (25 µg/h; 1.0 mL/h) dissolved in Ringer for 60 min. At 55' a bolus dose of BQ123 (100 µg/kg BW IV) Microspheres at 60'.

the glucose injection. During the ivGTT, arterial blood samples for analysis of insulin and CART levels in serum were collected at -30, 0, 5 and 30 min which were later analyzed by ELISA (Insulin, Mercodia and CART, Sigma Aldrich, Schnellendorf, Germany). Blood samples for CART analysis were preserved for measurements after a slight modification of the standardized method for measuring peptides developed by Hosoda et al. [29], i.e. collection in K₂ EDTA tubes, centrifugation at 5000X for 5 min at -4 °C and thereafter immediately stored in -80 °C.

2.4. Islet isolation and insulin release

Islets were isolated from Sprague-Dawley rats (M&B, Ry, Denmark) (n = 6 for each experiment) by collagenase digestion of the pancreas as previously described [30] and incubated in groups of around 100 islets for 48–72 h in RPMI 1640 cell medium (Sigma-Aldrich, R0883) supplemented with 10 % vol/vol fetal bovine serum (Sigma-Aldrich, F7524), 1 % vol/vol L-glutamine (Sigma-Aldrich, G7513) and 0,2 % vol/vol penicillin/Streptomycin (50 000 U/mL and 50 mg/ml respectively, 1074440001, Sigma-Aldrich). For the glucose stimulated insulin release (GSIS) islets were either incubated with CART 100 nmol/l or control media for one hour prior to the glucose stimulation (experiment

1, n = 6) or directly during the glucose stimulation by addition of CART 100 nmol/l to both the low- and high glucose media (experiment 2, n = 6). GSIS was performed with triplicates of ten islets from each animal per group (CART or control) incubated in Krebs-Ringer bicarbonate buffer supplemented with 10 mmol/l HEPES and 2 mg/ml bovine serum albumin (KRBH) under both low (1.67 mmol/l) and high glucose conditions (16.7 mmol/l) for one hour respectively. At the end of each step the media was collected, and the insulin concentration was analyzed by ELISA (Mercodia).

2.5. Statistical analysis

Calculations were performed using the statistical software Prism (GraphPad Software, San Diego, CA, USA). Parametric data with only two groups were analysed with Student's two-tailed t-test for unpaired and paired observations. Analysis of variance (ANOVA) with Bonferroni's post hoc test was used to compare multiple groups. For all comparisons, a P-value <0.05 was considered statistically significant. All values are expressed as means ± SEM.

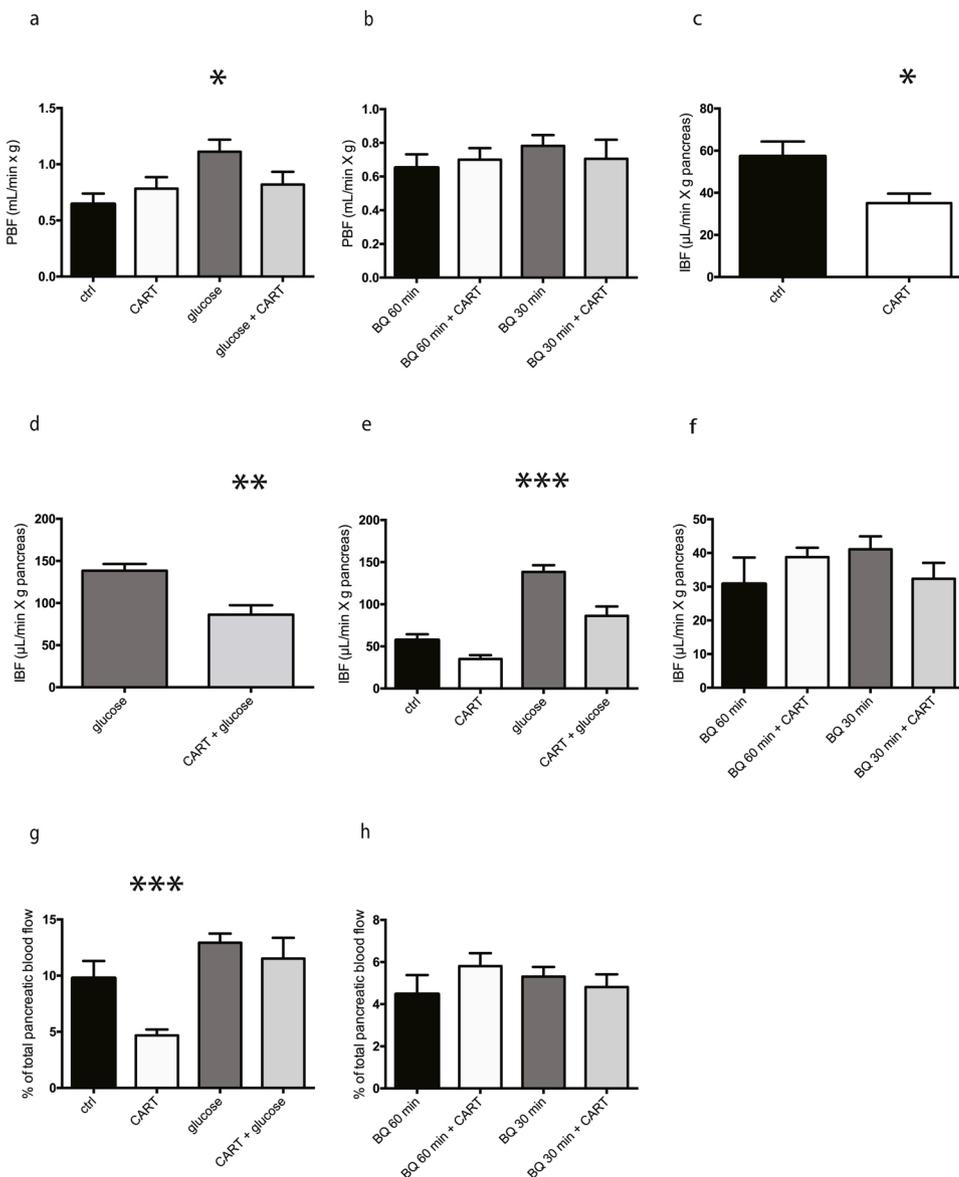


Fig. 1. (a) Pancreatic Blood Flow (PBF) (mL/min x g) in rats infused for 60 min with either saline (ctrl), CART, glucose or glucose + CART. No significant difference between groups was seen, except for glucose, a well-known PBF stimulator, n = 8 in all groups except 7 in the glucose + CART group, ANOVA, Bonferroni. P = < 0.05. (b) PBF in rats infused with BQ-123, either with, or without CART, infusion treatment for either 60 or 30 min, n = 7 in all groups, ANOVA, Bonferroni, p = 0.74 (c) Islet Blood Flow (IBF) (µL/min x g) in ctrl and CART infused rats, IBF is significantly decreased by CART, n = 8, students t-test, p = < 0.05 (d) IBF in rats infused with glucose followed by infusion of ctrl or CART, CART diminishes the expected IBF raise normally performed by glucose, n = 8 in the glucose group, and n = 7 in the glucose + CART group, students t-test, p = < 0.01 (e) IBF in rats infused with ctrl, or CART and in two groups infused with glucose followed by either ctrl- or CART infusion, figure (e) is a summary of all values in Figure (c) and (d), ANOVA, Bonferroni, p = < 0.001, n = 8, 8, 8 and 7 respectively (f) IBF in BQ-123 treated rats, either with or without CART, infusion treatment for either 60 or 30 min time, ANOVA, Bonferroni, p = 0.45, n = 7, 7, 8 and 8 respectively (g) IBF % of Pancreatic Blood Flow in ctrl, CART, glucose and glucose + CART infused rats, p = < 0.001, ANOVA, Bonferroni, n = 8, 8, 8 and 7 respectively (h) IBF % of Pancreatic Blood Flow in BQ-123 treated rats, either with or without CART, infusion treatment for either 60 or 30 min time, ANOVA, Bonferroni, p = 0.55, n = 7, 6, 8 and 8 respectively All values are +/- SEM, * denotes p = < 0.05, ** denotes p = < 0.01, *** denotes p = < 0.001, +/- SEM.

3. Results

3.1. CART concentrations and splanchnic blood flow

All animals maintained a mean arterial blood pressure of 100–120 mmHg during the *in vivo* experiments which was not affected by administration of either glucose, CART, BQ-123, BQ-123 together with CART or saline (data not shown). Plasma CART concentrations were higher in the group infused with CART (44.3 ± 2.7 pg/ml at 5 min in ivGTT) when compared with the control group (36.5 ± 2.4 pg/ml at 5 min in ivGTT, $p = 0.05$). The circulating CART levels increased during infusion of CART, while remaining unaffected in the control group. The circulating CART levels were unaffected also during the ivGTT, both in the CART and the control group (data not shown).

The PBF was similar in the control and CART group with or without simultaneous glucose administration, (Fig. 1a) and the addition of BQ-123 had no effect on the PBF (Fig. 1b). However, IBF was markedly decreased by CART infusion (Fig. 1c) and the increase in IBF normally induced by glucose was partly prevented by CART (Fig. 1d-e). BQ-123 over all decreased IBF selectively without an effect on total PBF, but there was no difference between the groups administrated BQ-123 alone or in combination with CART (Fig. 1f). The percentage of PBF diverted through the islets was similar in all groups, besides the group receiving CART in which the fractional IBF decreased (Fig. 1g-h). The duodenal-, colonic- and renal blood flow was not affected by CART or BQ-123, but glucose administration increased both the duodenal- and colonic blood flow (Suppl. Table 1).

3.2. Glucose tolerance tests

CART did not affect serum insulin concentrations during the ivGTT (Fig. 2a). The glucose tolerance as expressed as the glucose levels over time was also similar in the saline- and CART treated animals (Fig. 2b).

3.3. Insulin release

Pre-incubation of rat islets with CART (100 nmol/l) had no effect on insulin release during low (1.67 mmol/l) or high (16.7 mmol/l) glucose conditions when compared to control islets (Fig. 3a). In addition, there were no acute effects of CART on insulin secretion when added directly to the glucose media (low and high, Fig. 3b).

4. Discussion

CART is involved in a wide range of biological processes including feeding behavior, regulation of food intake, maintenance of body weight, reward and addiction [7], stress response, vascular regulation in the CNS [20,21] and insulin secretion [11,13,31]. In the present study we found that CART also affects the blood flow of pancreatic islets

without affecting the blood flow of the whole pancreas. Within the pancreas the local blood flow is regulated at the arteriolar level [32] in order to meet the various metabolic demands for hormonal secretion [33]. In addition to the direct effect of CART on IBF it also dampened the normal increase of IBF mediated by glucose. Despite the robust vasoconstricting effect of CART on IBF, we did not observe any acute effects on insulin secretion *in vivo* during basal- or glucose stimulated conditions as previously observed for ghrelin [34] and angiotensin II [35]. Similarly, we did not observe any effects of CART on insulin release from isolated pancreatic islets *in vitro*. However, it is possible that endogenous secretion of CART resulting in high local concentrations within the islets could exert such effects. Moreover, there may be differences between species regarding the pancreatic effects of CART [9]. In rats, CART augments insulin secretion at high glucose-levels, when amplified by cAMP and the Protein kinase A (PKA) dependent pathway [11,31]. On the other hand, one previous study has in contrast to our findings, found that CART inhibits insulin secretion in response to high glucose [11]. Hence, in rats, CART either stimulates insulin secretion if cAMP levels are high, whilst it seems as if CART under basal cAMP levels has either no effect or an inhibitory effect on insulin secretion, suggesting that the possible stimulatory effect by CART is mediated by PKA [11,31,36]. In mice and humans CART has been found to exert a stimulatory effect on insulin secretion [9,12]. In part this could be explained by the different pattern of CART expression within the pancreatic islets between the different species. Therefore, the result in rat is not necessarily translatable to human. At the same time CART inhibits glucagon secretion in islets in a glucose-dependent fashion [12]. The glucagon inhibiting effect is of supplemental interest since only a few hormones have been ascribed this effect, i.e. insulin [37], somatostatin [38] and GLP-1 [39].

Given the technical difficulties associated with measuring IBF and the invasive nature of the method, rats are the most widely used animal model for studies of IBF. In the present study, no cAMP elevating agents were used which may be a subject for additional further studies.

Previous publications have reported that the circulating plasma concentration of CART is in the range of 10–180 pmol/l [10,19,40] and in combination with the marked physiological effect on IBF we conclude that our selected dose of CART (5000 nmol/l *in vivo* and 100 nmol/l *in vitro*) is potent enough. Due to limitations of microsphere access from deliverer, only one dose could be investigated in the present study and therefore we were not able to study the kinetics of CART in detail.

Despite that there are numerous studies on the physiological effects of CART its receptor is still unknown. A possible explanation is that CART could be promiscuous in its nature and act through several different receptors either directly or via mediated effects. If so, this could explain some of the contradictory effects ascribed to CART in different publications. It has, for instance, been proposed that CART potentially can act through a G protein-coupled receptor [25], which would suggest an inhibition of adenylate cyclase (G- $\alpha_{i/o}$) [26] or stimulation of

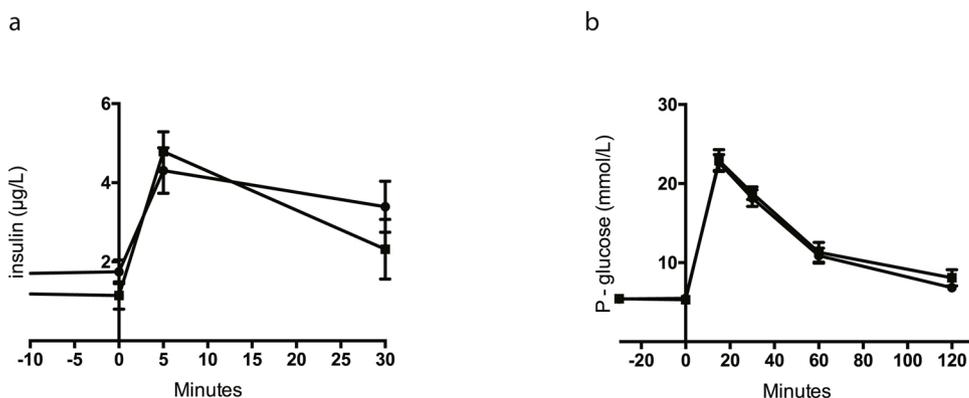


Fig. 2. (a) Insulin levels during *i.v.* Glucose Tolerance Test (ivGTT) and infusion of either saline or CART, $n = 9$, no significant difference at any time point tested through separate student *t*-tests, p -value = 0.54 at the 5 min comparison. (b) Glucose levels during ivGTT in saline and CART infused rats, $n = 9$, no significant difference between groups, p -value at 15 min = 0.89, (ctrl = closed circles, CART = closed squares) in both (a) and (b) All values are +/- SEM.

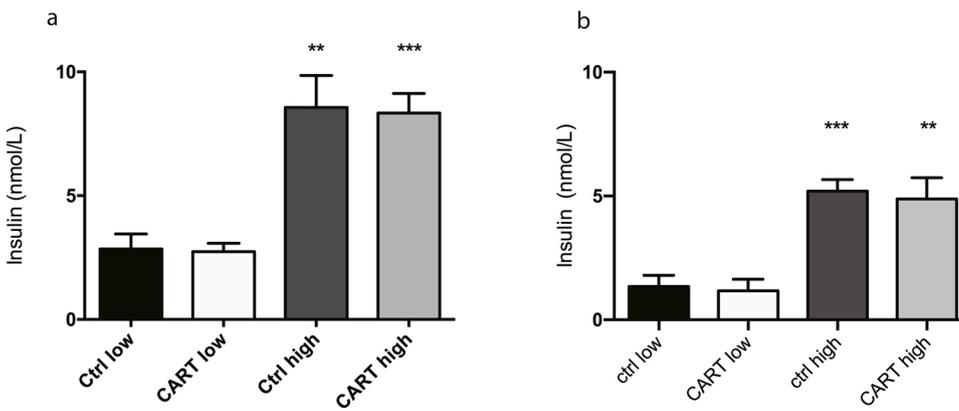


Fig. 3. (a) Insulin supernatant concentrations after insulin release of triplicates of 10 isolated islets, either incubated with or without CART (100 nmol/L) preceding insulin release, incubation at basal low glucose (1.67 mmol/L) concentration for one hour (**left side**) and high glucose-stimulated (16.7 mmol/L) concentration for a second hour (**right side**). (t-tests were performed between groups, p-value 0.89 for low and 0.88 for high value comparison between groups, p-value = < 0.01 for ctrl, low and high, and = < 0.001 for CART, low and high comparison).

All values are +/- SEM for islets from 6 animals. (b) Insulin supernatant concentrations after insulin release of triplicates of 10 isolated islets, either incubated with or without CART (100 nmol/L). CART incubation is performed during glucose stimulated insulin release (GSIS), incubation at basal low glucose (1.67 mmol/L) concentration for one hour (**left side**) and high glucose-stimulated (16.7 mmol/L) concentration for a second hour (**right side**), (t-tests are performed between groups, p-value 0.79 for low and 0.75 for high value comparison, p-value = < 0.001 for ctrl, low and high, and = < 0.01 for CART, low and high comparison)

All values are +/- SEM for islets from 6 animals.

G α s-coupled receptor in beta cells [41]. These theories could further support that an addition of a cAMP elevating agent would be an interesting next procedure in evaluating the mechanism through which CART performs its action.

ET-1 has been proposed as an important mediator of the direct constriction of vascular tone by CART [20,42], and Illif et al. found that the administration of ET-1 antagonists (nonspecific PD-145,065 and ET_A specific BQ-123) blocked the vasoconstrictive effect of CART in cerebral arteries in rats while the ET_B-specific antagonist (BQ-788) had no effect on the vascular response to CART [20]. Therefore, we examined the combination of CART and BQ-123 but this combination did not significantly change the PBF or IBF, compared to CART or BQ-123 alone. These differences could at least partly be explained by the findings by Lai et al. [23], where the constricting effect by ET-1 could not be prevented by either ET_A or ET_B antagonists alone or in combination [43]. In summary, the reduction of IBF by CART observed in the present study is highly suspected to be mediated via a direct effect of CART since CART alone, and even in combination with glucose, had an inhibiting effect on IBF. However, the effect does not seem to be mediated by an interaction with ET-1, and thus the specific mechanism of action of CART on the pancreatic islet vasculature still remains unknown.

There are a number of factors known to increase IBF in response to an increased metabolic demand, for instance glucose [27], tolbutamide [44] and glucagon [43]. However, the factors that act on the local pancreatic vasculature in order to reduce IBF in response to a decreased metabolic demand for insulin secretion, such as hypoglycaemia, are still not well described [45,46]. We have recently found that the islet hormone ghrelin specifically decreases IBF [34]. In the present study, we found that CART also decrease IBF, both under basal and glucose stimulated conditions. Despite the marked decrease in IBF, neither mean arterial blood pressure, PBF or intestinal blood flow was affected by exogenous CART administration, suggesting a specific effect on the islet vascular bed.

Under normal physiological conditions IBF accommodates the metabolic demands and is regulated to meet the need for insulin secretion [45,47]. Although, we did not observe any acute effects of CART on insulin secretion under basal or glucose stimulated conditions this does not exclude the possibility that a chronic increase of circulating CART levels could negatively impact the pancreatic islets. In addition,

the expression of CART has been found to be increased in vivo and in islets of type 2 diabetic humans and in islets of rodents [12]. Given that CART has been ascribed both a glucose dependent insulinotropic effect and inhibitory effects on glucagon secretion [12] its effects on pancreatic islets seems to largely mimic those of incretins. However, incretins have previously been shown to potentiate glucose-stimulated IBF [48]. It could be speculated that the restricting effect of CART on IBF and hampered increase of IBF in response to glucose could potentially represent a previously unknown compensatory mechanism aiming to protect pancreatic islets from the increasing shear stress and nutrient overload during the progression of diabetes.

In conclusion, we report on a potent and specific vascular constricting effect by CART on pancreatic IBF. To the best of our knowledge, there are no previous reports on the specific local effects of CART on IBF. Further studies are indicated in order to delineate the underlying mechanisms and receptor through which CART exerts its vasoconstrictive actions and the potential impact in the development of beta cell failure in diabetes.

Funding

This study was supported by grants from the Swedish Society for Medical Research, EXODIAB and Barndiabetesfonden.

All funding sources have no involvement or role in any details of this study.

CRediT authorship contribution statement

Carl Johan Drott: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Daniel Norman:** Methodology, Validation, Formal analysis, Software, Investigation, Writing - review & editing. **Daniel Espes:** Conceptualization, Methodology, Investigation, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors have nothing to disclose.

Acknowledgments

The highly skilled technical assistance from Birgitta Bodin and Lisbeth Ahlqvist, Department of Medical Cell Biology, Uppsala University, and valuable discussion with professor Leif Jansson, Department of Medical Cell Biology, Uppsala University, are gratefully acknowledged.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.peptides.2020.170431>.

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