

Inhibitory effect of somatostatin on insulin secretion is not mediated via the CNS

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Abstract

The inhibitory effect of somatostatin (SST) on insulin secretion *in vivo* is attributed to a direct effect on pancreatic beta cells, but this is inconsistent with some *in vitro* results in which exogenous SST is ineffective in inhibiting secretion from isolated islets. We therefore investigated whether insulin secretion from the pancreatic islets may partly be regulated by an indirect effect of SST mediated via the CNS. Islet hormone secretion was assessed *in vitro* by perfusion and static incubations of isolated islets and *in vivo* by i.v. or i.c.v. administration of the SST analogue BIM23014C with an i.v. glucose challenge to conscious, chronically catheterised rats. Hormone content of samples was assessed by ELISA or RIA and blood glucose levels using a glucose meter. Exogenous SST14/SST28 or BIM23014C did not inhibit the release of insulin from isolated rodent islets *in vitro*, whereas peripheral i.v. administration of BIM23014C (7.5 µg) with glucose (1 g/kg) led to decreased plasma insulin content (2.3 ± 0.5 ng insulin/ml versus 4.5 ± 0.5 ng/ml at $t=5$ min, $P < 0.001$) and elevated blood glucose levels compared with those of the controls (29.19 ± 1.3 mmol/l versus 23.5 ± 1.7 mmol/l, $P < 0.05$). In contrast, central i.c.v. injection of BIM23014C (0.75 µg) had no significant effect on either plasma insulin (3.3 ± 0.4 ng/ml, $P > 0.05$) or blood glucose levels (23.5 ± 1.7 mmol/l, $P > 0.05$) although i.v. administration of this dose increased blood glucose concentrations (32.3 ± 0.7 mmol/l, $P < 0.01$). BIM23014C did not measurably alter plasma glucagon, SST, GLP1 or catecholamine levels whether injected i.v. or i.c.v. These results indicate that SST does not suppress insulin secretion by a centrally mediated effect but acts peripherally on islet cells.

Key Words

- ▶ somatostatin
- ▶ CNS
- ▶ insulin secretion
- ▶ intracerebroventricular

Journal of Endocrinology
(2015) 225, 19–26

Introduction

Somatostatin (SST) is synthesised and secreted by neuroendocrine cells in the CNS and in the gastrointestinal and immune systems (Reichlin 1983a,b). In the pancreatic islet, the peptide is produced by δ -cells, which comprise approximately 5% of the islet endocrine cells. SST exists as two biologically active forms, SST14 and SST28, which are derived by post-translational cleavage of a single

prohormone. SST14 is expressed in neurones and most other SST-producing peripheral tissues, including δ -cells, while SST28 is primarily found at high levels in the gastrointestinal tract (Low 2004), and is the major form of circulating SST (Polonsky *et al.* 1983). The peptide often acts as an inhibitory regulator of endocrine systems, for example, as a hypothalamic factor to suppress growth

hormone secretion, or as a local inhibitor of the release of gastrointestinal peptide hormones. The effect of SST is mediated through binding to SST receptors of which five different isoforms have been identified (reviewed by Moller *et al.* (2003)). In pancreatic islets, receptors have been identified on both α - and β -cells (Strowski *et al.* 2000, Cejvan *et al.* 2003) and exogenously administered SST inhibits both insulin and glucagon secretion (Schuit *et al.* 1989, Strowski *et al.* 2000, Cejvan *et al.* 2003), findings consistent with a negative regulatory role for SST in islet secretory function. In rodents, this effect is primarily mediated via receptors 5 and 2, respectively, although all five receptors are expressed in the islets (Ludvigsen *et al.* 2004). Results from studies using SSTR-deficient mice (SSTR1, SSTR2 or SSTR5) revealed changes in both basal and stimulated insulin secretion (Strowski *et al.* 2000, Wang *et al.* 2005, 2006), which is consistent with a role for SST in regulating hormone release. We have recently used islets from SST-deficient mice to demonstrate that SST released from the δ -cell exerts a tonic inhibitory input on hormone secretion from both α - and β -cells (Hauge-Evans *et al.* 2009) and it has further been shown that the release of islet SST, like insulin, is stimulated by glucose (Vieira *et al.* 2007, Hauge-Evans *et al.* 2012).

SST and SST analogues inhibit insulin secretion when administered *in vivo* (Lins *et al.* 1980) and this response to exogenous SST is generally attributed to a direct inhibitory effect on the islet β -cells. There are, however, some inconsistencies in the literature regarding the effectiveness of SST *in vitro*. Thus, whereas the results of some studies indicate that SST inhibits stimulus-dependent hormone release from isolated rodent and human islets as well as the perfused pancreas (e.g. Casad & Adelman 1992, Zambre *et al.* 1999, Strowski *et al.* 2000), others have found that exogenous SST was ineffective in suppressing insulin or glucagon secretion from isolated mouse islets (Cejvan *et al.* 2003, Hauge-Evans *et al.* 2009). An analogous system has been reported in the exocrine pancreas, where exogenous SST did not inhibit enzyme secretion from isolated, arterially perfused pancreas, nor from acinar cells *in vitro*, but suppressed pancreatic enzyme release indirectly via binding to SSTR2 in the dorsal vagal complex (Liao *et al.* 2007).

These observations imply the existence of an additional mechanism through which exogenous SST can inhibit insulin secretion *in vivo*, and as all five SST receptors are widely expressed throughout the brain (Moller *et al.* 2003), we proposed an indirect, central effect of SST, potentially mediated by the sympathetic nervous system. We have therefore assessed the effect on insulin and glucagon secretion of i.v. or i.c.v. administration of BIM23014C,

a broad-range SSTR agonist also known as Lanreotide, in parallel with an i.v. glucose challenge in rats.

Methods

Islet isolation

Islets were isolated from male Wistar rats or ICR mice (Harlan, Bicester, UK) by collagenase digestion (1 mg/ml, type XI, Sigma) and separated from exocrine pancreatic tissue on a histopaque gradient (Sigma), as described previously (Hauge-Evans *et al.* 2009). The islets were incubated overnight at 37 °C (5% CO₂) in RPMI 1640 (10% FBS, 2 mmol/l glutamine, 100 units/ml penicillin/0.1 mg/ml streptomycin and 11 mmol/l glucose) before experiments were performed.

In vitro hormone secretion studies

For measuring the dynamics of insulin release, islets were transferred into chambers of a temperature-controlled multi-channel perfusion system as described previously (Jones *et al.* 1989) and perfused for 70 min at 37 °C with a low-glucose, bicarbonate-buffered physiological salt solution (Gey & Gey 1936). The tissue was subsequently perfused (0.5 ml/min) with salt solution containing agents of interest and fractions were collected at 2 min intervals. For static secretion experiments, islets were pre-incubated for 60 min in a buffer containing 1 mmol/l glucose after which batches of eight islets were incubated for 60 min in 0.4 ml salt solution containing agents of interest.

Animals and surgical procedures for *in vivo* studies

Adult male Wistar rats (approximately 300 g, Harlan) were housed under controlled conditions and provided with food and water and allowed to eat and drink *ad libitum*. All animal procedures were undertaken in accordance with the UK Home Office Regulations. All surgical procedures were carried out under anaesthesia induced with ketamine (100 mg/kg i.p.; Pharmacia and Upjohn Ltd, Crawley, UK) and Rompun (10 mg/kg i.p.; Bayer). Rats were fitted with a guide and a dummy cannula directed towards the left lateral cerebral ventricle, as described previously (Bowe *et al.* 2008) and, following a 10-day recovery period, the animals were fitted with two indwelling cardiac catheters via the external jugular veins. The catheters were exteriorised at the back of the head and secured to a cranial attachment and the animals were fitted with a 30-cm long metal spring tether, which was

attached to a fluid swivel, allowing free movement of the rats in their enclosure. Experiments were commenced 3 days later.

In vivo experimental procedure

Before experimental tests, the rats were fasted overnight. On the day of the experiment, conscious rats received injections either i.c.v. with physiological saline or with the SSTR agonist, BIM23014C (Lanreotide acetate, Sigma), dissolved in physiological saline (0.75 µg) or i.v. with BIM23014C (7.5 µg) in parallel with i.v. administration of glucose (1 g/kg) at time point 0. Blood samples were withdrawn with heparinised syringes via cardiac catheters at -10, -5, 0, 5, 10, 20, 30, 45 and 60 min following BIM23014C and glucose administration at a volume of 100 µl except at time points -5, 10 and 30 min, where 300 µl were drawn. Blood glucose concentration was measured using a glucose meter (Accucheck Aviva, Roche) after which the plasma was separated by centrifugation (2351 g, 5 min at 4 °C) and frozen for later assay of islet hormone content. Each treatment group contained two animals per experimental day and the experiment was repeated every 3–4 days with animals exposed to different treatments each time to give a total number of 4–11 per treatment group.

Hormone immunoassays

The islet hormone and catecholamine content of plasma samples were assessed by ELISA, using commercially available kits (Insulin: Millipore Ltd, Watford, UK; glucagon:

Mercodia, Uppsala, Sweden; SST and GLP1: USCN Life Science, Inc., Wuhan, China; catecholamine: Bioassay Technology Laboratory, Shanghai, China). Islet hormone concentrations in the incubation medium from *in vitro* experiments were assessed by RIA using an in-house insulin assay as described previously (Hauge-Evans *et al.* 2009).

Statistical analysis

Data are expressed as means ± s.e.m. and analysed using Student's *t*-test or one- or two-way ANOVA as appropriate and Bonferroni's multiple comparisons test. The choice of test is indicated in the figure legends. Differences between treatments were considered significant at $P < 0.05$.

Results

We have previously shown that exogenous SST14 had little inhibitory effect on dynamic insulin secretion from isolated mouse islets in response to 20 mmol/l glucose (Hauge-Evans *et al.* 2009), and herein we confirm these findings under a range of conditions. These include intermediate and high concentrations of both glucose and SST14 in static and dynamic experimental settings for measurements of insulin release as well as with isolated islets from a different species, the rat (Figs 1 and 2). No effect on dynamic insulin release was observed, whether islets were exposed to SST14 after an initial stimulation by glucose (Fig. 1A), or whether they were co-perfused with glucose and SST14 simultaneously (Fig. 1B and C). In parallel experiments, SST28 was similarly ineffective in modulating dynamic glucose-induced

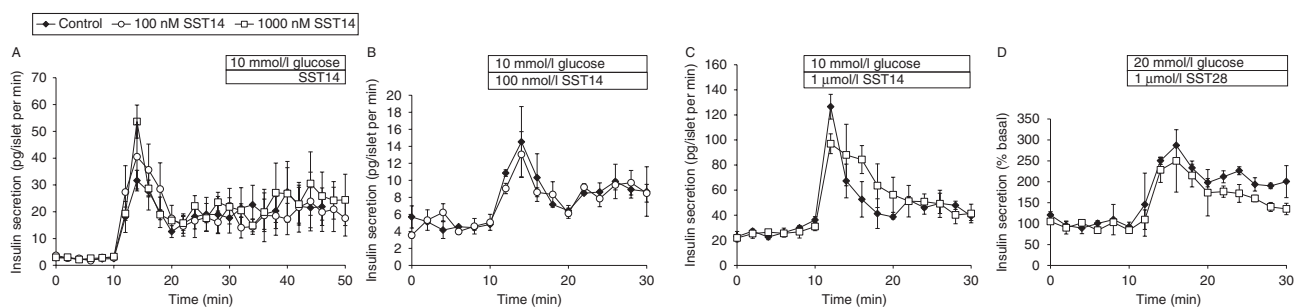
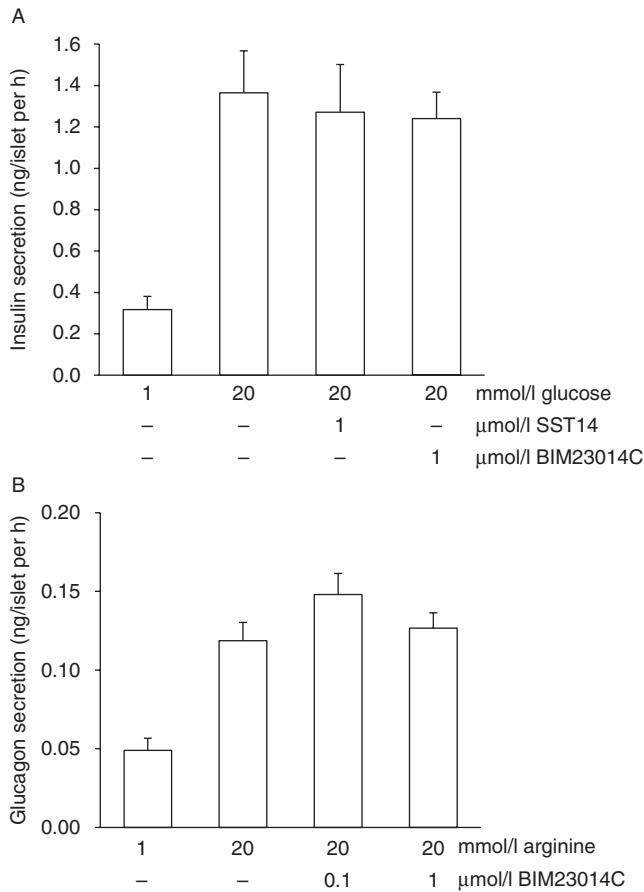


Figure 1

No effect of exogenous somatostatin on dynamic insulin secretion from isolated rodent islets. (A) Following perfusion with a sub-stimulatory concentration of glucose (2 mmol/l, 0–10 min), mouse islets were stimulated with an intermediate glucose concentration (10 mmol/l) with or without 100 nmol/l or 1 µmol/l SST14 either following (30–50 min, A), or during (10–30 min, B and C) the initial glucose challenge. None of the concentrations of SST14 had any inhibitory effects on dynamic insulin

secretion. In D, islets were co-perfused with 20 mmol/l glucose with or without 1 µmol/l SST28. Points show means ± s.e.m. for two to four separate perfusion channels. Data were analysed statistically by comparing the AUC of insulin release during SST treatment with that of controls using Student's *t*-test or one-way ANOVA followed by Bonferroni's post-test. $P > 0.05$ for all comparisons.

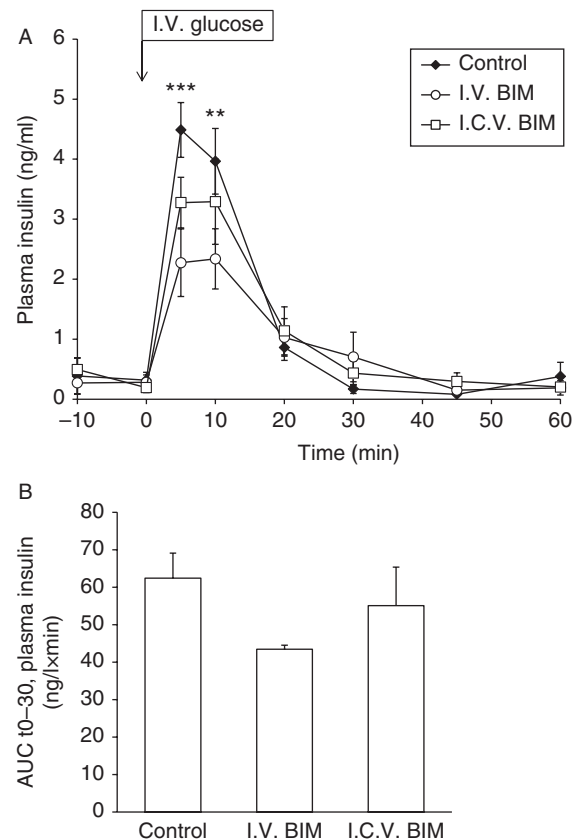
**Figure 2**

No effect of exogenous somatostatin on static insulin or glucagon secretion from isolated rodent islets. In static incubations of rat islets, exogenous SST14 (1 $\mu\text{mol/l}$) or the stable SST analogue, BIM23014C (1 $\mu\text{mol/l}$), did not inhibit glucose-induced insulin release (A) nor did BIM23014C reduce arginine-induced glucagon secretion from mouse islets (B). Bars show means \pm s.e.m. from one representative experiment ($n=7-8$). $P>0.05$ as assessed by one-way ANOVA followed by Bonferroni's post-test.

insulin secretion from isolated rodent islets (Fig. 1D) as was the stable SST analogue, BIM23014C (Fig. 2A). In addition, BIM23014C did not inhibit arginine-induced glucagon secretion (Fig. 2B).

To assess whether SST exerts an effect on insulin release indirectly via a centrally mediated mechanism, the SST analogue, BIM23014C, was injected either peripherally (i.v.) or centrally (i.c.v.), the latter at a tenfold lower dose to compensate for the smaller volume of the ventricular system compared with the systemic circulation. Assuming approximate blood and cerebrospinal fluid (CSF) volumes of distribution of 20 ml and 300 μl , respectively, the predicted concentrations of BIM23014C in blood and CSF will be in the order of 300 nmol/l and 2 $\mu\text{mol/l}$ respectively. This is well above that required to activate SST receptors,

including those less preferentially targeted by the agonist (Meyerhof 1998) and similar to doses used in other studies (Rettig *et al.* 1989, Karasawa *et al.* 2014). As shown in Fig. 3A, i.v. administration of BIM23014C at $t=0$ together with glucose (1 g/kg) led to an immediate and significant decrease in plasma insulin levels in response to the glucose challenge compared with controls with saline and glucose only, and this decrease was paralleled with significantly increased blood glucose levels (Fig. 4A and B). The effect of i.v. BIM23014C on insulin release was observed primarily during the initial 10 min of the response and was reflected in a decreasing trend of AUC for 30 min compared with control as shown in Fig. 3B. Despite the stable nature of the compound (half-life: 90 min), there was no difference in

**Figure 3**

Peripheral (i.v.) vs central (i.c.v.) effects of the SST analogue, BIM23014C, on plasma insulin. Time course (A) and AUC (B) of plasma insulin levels in rats following i.v. or i.c.v. injection of BIM23014C (7.5 and 0.75 μg respectively) at the same time ($t=0$) as an i.v. glucose bolus (1 g/kg) as indicated by the arrow. While i.v. administration of the SST analogue significantly reduced the initial peak of plasma insulin (0–20 min) compared with controls (saline and glucose only), i.c.v. administration had no significant effect. $n=4$ animals per treatment group. $**P<0.01$, $***P<0.001$, i.v. versus control as assessed by two-way (A) or one-way (B) ANOVA followed by Bonferroni's post-test.

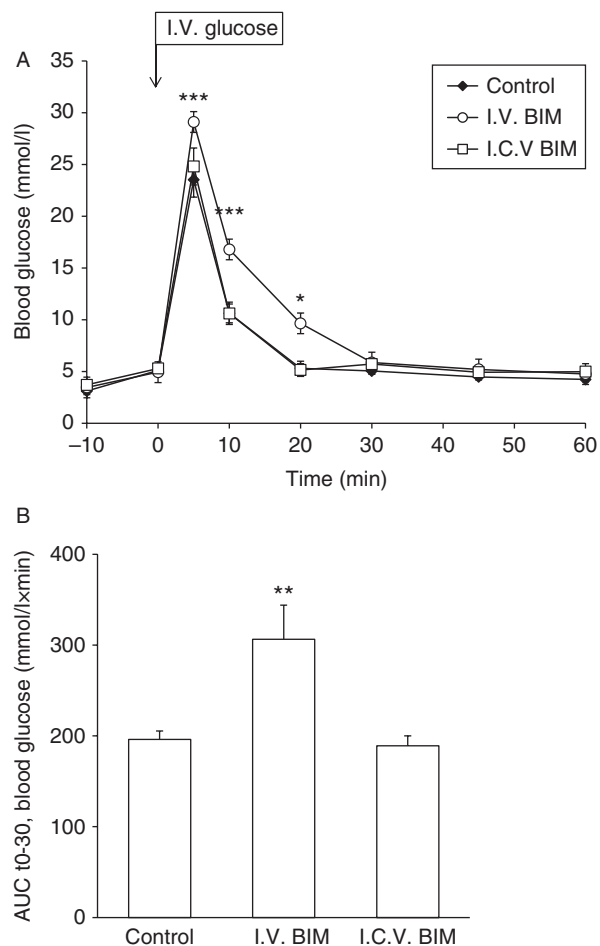


Figure 4

Peripheral (i.v.) vs central (i.c.v.) effects of the SST analogue, BIM23014C, on blood glucose concentrations. Time course (A) and AUC (B) of blood glucose levels following i.v. or i.c.v. administration of BIM23014C at the same time as an i.v. glucose bolus (1 g/kg, $t=0$) as shown in Fig. 3. Consistent with plasma insulin levels, blood glucose concentrations were increased following i.v. injection but not i.c.v. injection of BIM23014C compared with controls (saline and glucose only). $n=9-11$ animals per treatment group. *** $P<0.001$, ** $P<0.01$, * $P<0.05$ i.v. versus control as assessed by two-way (A) or one-way (B) ANOVA followed by Bonferroni's post-test.

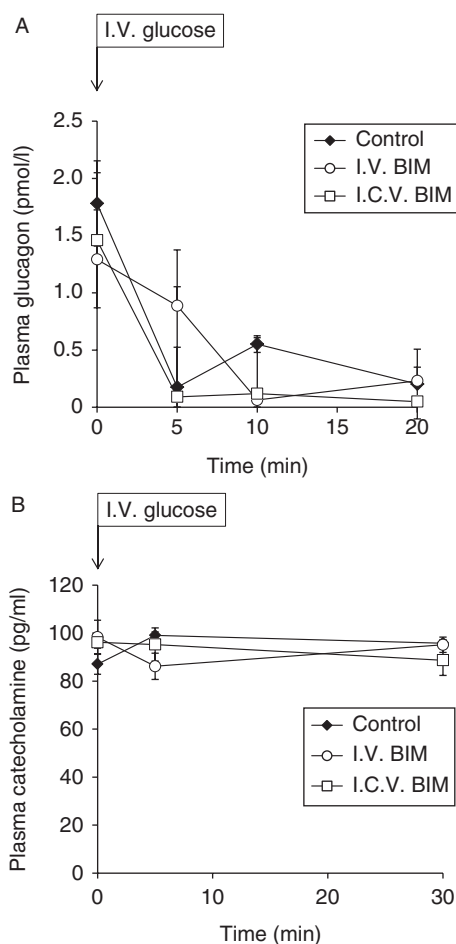
plasma insulin levels compared with controls once the elevated blood glucose concentrations had been cleared from the circulation ($P>0.2$ for $t=60$ and 120 min). In contrast to peripheral administration of BIM23014C, i.c.v. injection of the compound at a high central concentration had no significant effect on the time course of insulin release or the overall AUC (Fig. 3A and B). Accordingly, no parallel changes in blood glucose concentration were observed when BIM was administered simultaneously with- (Fig. 4A and B) or 5 min before i.v. glucose injection (control AUC: 195.7 ± 14.3 mmol/l \times min; i.c.v. BIM23014C and i.v. glucose co-treatment: 186.9 ± 24.1 mmol/l \times min; i.c.v. BIM23014C pre-treatment:

126.2 ± 8.8 , $n=3-4$, $P>0.05$). In contrast, i.v. administration of the same dose ($0.75 \mu\text{g}$) led to increased blood glucose levels compared with controls (AUC: 331.3 ± 32.7 mmol/l \times min versus 193.1 ± 20.9 mmol/l \times min, $P<0.05$). These results thus indicate that central activation of SSTRs by the SST analogue had no modulatory effect on insulin release from the pancreas and that the i.v. effect of the SST analogue was therefore unlikely to be mediated indirectly by a CNS-dependent mechanism.

Glucagon is a major regulator of glucose homeostasis and both glucose and SST inhibit glucagon secretion from α -cells within the islet. Results from the assessment of plasma glucagon levels following i.v. or i.c.v. administration of BIM23014C together with i.v. glucose indicated that the compound under either experimental setting did not further repress the already very low levels of plasma glucagon, as shown in Fig. 5A. These results were further confirmed in a second experimental cohort, where no significant difference was observed between either of the three treatment groups at time points 0, 5 and 30 min ($n=6$ animals per group, $P>0.05$). In addition, there were no significant changes in plasma catecholamine levels between treatment groups at these time points (Fig. 5B, $P>0.05$), indicating that the observed increases in blood glucose levels following i.v. BIM23014C administration were a consequence of changes in plasma insulin levels rather than a potential indirect effect of BIM23014C on glucagon release mediated by changes in levels of circulating catecholamines. Consistent with the route of administration, i.v. injection of glucose did not affect the plasma content of GLP1 or SST at the 10 or 30 min time points and i.v. or i.c.v. BIM23014C did not further alter the basal levels of these hormones (results not shown).

Discussion

The effect of SST on the secretion of peptides from the exocrine pancreas and on intestinal uptake of water and electrolytes is mediated indirectly via the CNS (Primi & Bueno 1987, Li & Owyang 1993, Liao *et al.* 2007) and other islet hormones have similarly been shown to convey their effects indirectly via the CNS in addition to their direct action on target tissues. For example, insulin acts indirectly via insulin receptors expressed on the ventromedial hypothalamus to modulate peripheral glucose homeostasis in addition to its direct action on target tissues (Diggs-Andrews *et al.* 2010, Paranjape *et al.* 2010). We therefore proposed the hypothesis that the inhibitory effect of SST on insulin release may similarly be at least partly dependent on central activation of SSTRs by SST.

**Figure 5**

Plasma glucagon and catecholamine levels following peripheral or central administration of the SST analogue, BIM23014C. (A) i.v. glucose injection (1 g/kg at $t=0$) led to a decrease in overall plasma glucagon levels, which were not further affected by i.v. or i.c.v. administration of BIM23014C (7.5 μg and 0.75 μg respectively). $n=4$ animals. These results were confirmed using a second cohort of animals ($n=6$, $P>0.05$, control versus i.v. or i.c.v. BIM23014C as assessed by two-way ANOVA). (B) Circulating catecholamine concentrations were not significantly affected by i.v. glucose administration in the absence (control) or presence of simultaneous administration of BIM23014C by the i.v. or i.c.v. route ($P>0.05$ by two-way ANOVA, $n=6$).

To test our theory, the SST analogue, BIM23014C, was administered either i.v. or i.c.v. The i.c.v. injection of compounds into the ventricular system by is a well-established method and was chosen because it allows for several brain regions to be affected simultaneously, rather than limiting the target area to one specific part of the brain. While i.v. injection of the compound gave the expected decrease in plasma insulin and a concomitant increase in blood glucose levels consistent with reduced insulin action on target tissues, no significant effects were observed following infusion via the i.c.v. route, despite the

estimated local concentration being higher after i.c.v. than after i.v. injection due to the much smaller distribution volume of the CSF (Pheng *et al.* 2009). The use of a lower overall dose i.c.v. versus i.v. also minimised the likelihood of leakage of BIM23014C into the peripheral circulation by clearance from the CSF, which could mask a central action of the compound. The rationale behind this approach was supported by findings by Tannenbaum & Patel (1986), indicating that centrally administered SST affected not only GH secretion, but also increased basal blood glucose levels. However, this was attributed to measurable leakage of SST from the cerebral ventricles into the general circulation, which could be measured peripherally within minutes of i.c.v. administration of high peptide concentrations. Based on the dosage used in this study, this was unlikely to be the case in our experiments, and we saw no effect of i.c.v. BIM23014C on basal glucose levels, although i.v. administration of the same dose led to an increase in blood glucose concentrations following i.v. glucose administration when compared with controls.

Results from our previous studies with SST KO mice indicated that SST primarily affects insulin release under stimulatory conditions (Hauge-Evans *et al.* 2009) and i.v. administration of glucose enabled us to study this with minimal changes to incretin hormone and endogenous SST levels from the gastrointestinal tract otherwise observed following oral or intraduodenal glucose administration. This is relevant as both affect overall plasma insulin levels. We did not observe any significant change in the incretin hormone GLP1 or plasma SST 10 min after i.v. glucose injection, consistent with the route of administration, and BIM23014C administration (i.v. or i.c.v.) had no significant effect on their basal levels, indicating that the influence of peripheral factors from the intestine was limited in the present experimental protocol.

Our results indicated that i.v. glucose administration led to immediate changes in both plasma glucose and insulin levels and we have previously reported that this takes place within 2–3 min of injection (Bowe *et al.* 2014), confirming an instantaneous response of pancreatic β -cells to hyperglycaemia, which in this study was promptly modulated by i.v. administration but not by i.c.v. administration of BIM (within 5 min). In contrast, Yavropoulou *et al.* (2014) reported that bolus i.c.v. infusion of SST14 with a simultaneous and continuous intraduodenal glucose infusion in dogs caused a delayed reduction of both plasma insulin and glucose levels 45–90 min after administration despite the very short plasma half-life of SST14 (2–3 min). It seems likely that the different experimental outcomes reflect differences in the experimental protocols, in particular with

regard to the route of glucose administration, where a local incretin effect as well as a centrally mediated effect of SST on the intestine cannot be excluded. Under conditions where intestinal influences are minimised, as in our experimental model, we did not observe any significant changes in plasma insulin and blood glucose levels in response to a locally high i.c.v. BIM23014C concentration.

In summary, while measurement of glucose homeostasis by i.v. administration cannot be used to rule out a subtle, long-term effect of i.c.v. SST under physiological circumstances, this study using conscious, unrestrained experimental animals could find no evidence for an important centrally mediated effect of SST in regulating glucose-induced insulin secretion *in vivo*, although we cannot exclude the possibility of the presence of other indirect, but peripheral pathways mediating the effect of SST on islet endocrine cells to suppress hormone secretion. Given the discrepancies within the literature regarding an inhibitory action of SST *in vitro*, it is also possible that the lack of effect of exogenous SST or SST analogues in suppressing glucose-induced insulin (and glucagon) release from isolated islets *in vitro* is a consequence of islet isolation and/or culture, perhaps through alterations in SST receptor expression or via changes in the tonic inhibitory input from islet δ -cells. Whatever the underlying cause(s), the very different effect of SST on insulin secretion between *in vivo* and *in vitro* experimental models is a potential confounding factor worth considering when designing and interpreting studies in this area.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This work was funded by Diabetes UK and ACHE is a Diabetes UK RD Lawrence Fellow (BDA: 10/0003980).

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Received in final form 23 January 2015

Accepted 28 January 2015

Accepted Preprint published online 28 January 2015