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BTS 67 582 stimulates insulin secretion from perifused rat pancreatic islets

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Abstract

The novel antidiabetic agent BTS 67 582 (1,1-dimethyl-2-[2-(4-morpholinophenyl)]guanidine monofumarate) demonstrated a concentration-dependent stimulation of insulin release in perifused rat pancreatic islets. EC_{50} values of 7.7 μM and 6.3 μM were obtained for BTS 67 582 in the presence of 8 mM glucose, after islets were pre-equilibrated with 4 and 8 mM glucose respectively. In contrast, there was little or no stimulation of insulin release at substimulatory (4 mM) or maximal stimulatory (15 mM) glucose concentrations. The plasma EC_{50} value for the glucose lowering effect of BTS 67 582 in fasted normal rats was 3.9 μM indicating a similar potency in vivo. In islets, BTS 67 582 completely antagonised (EC_{50} value of 13.2 μM) the actions of the selective ATP-dependent K^+ channel opener diazoxide indicating K^+ channel blocking activity. BTS 67 582 only weakly reversed the α_2-adrenoceptor mediated inhibition of insulin release in islets (EC_{50} of 83 μM). BTS 67 582, like other imidazoline/guanidine insulin releasing agents, appears to promote insulin release via an effect on the islet ATP-dependent K^+ channel which is not mediated by binding to the sulphonylurea receptor. © 1997 Elsevier Science B.V.

Keywords: BTS 67 582; Islets of Langerhans; Insulin release; Imidazoline receptor; Diabetes

1. Introduction

BTS 67 582 (1,1-dimethyl-2-[2-(4-morpholinophenyl)]guanidine monofumarate) is a novel glucose lowering agent currently in phase II clinical trial for the treatment of non insulin dependent diabetes mellitus (NIDDM, Byrom et al., 1996; Skillman and Raskin, 1997). Previous work has shown that BTS 67 582 lowers blood glucose concentrations in both normal and streptozotocin-induced diabetic rats with a concomitant increase in plasma insulin concentration (Jones et al., 1997). The same study also showed that BTS 67 582 did not displace [^{3}H] glibenclamide from its binding site in various membrane preparations. Preliminary work has shown that BTS 67 582 can inhibit the ATP-dependent K^+ channel in patch clamped membranes from pancreatic β-cells (Jones et al., 1996). This work suggests that BTS 67 582 is probably acting as an insulin releasing agent by depolarising the pancreatic β-cell membranes via a non sulphonylurea receptor mediated inhibition of the ATP-dependent K^+ channel. There have, however, only been preliminary in vitro studies demonstrating that the compound can cause insulin secretion (Dunne et al., 1995; Dickinson et al., 1997).

In this study the effects of BTS 67 582 in perifused pancreatic islets from normal rats have been characterised. BTS 67 582, in islets, acts to potentiate the effect of glucose with minimal effects at non stimulatory and maximal stimulatory glucose concentrations. BTS 67 582 reversed the diazoxide mediated inhibition of insulin secretion suggesting activity at the ATP-dependent K^+ channel. BTS 67 582 is only a weak functional α_2-adrenoceptor antagonist in islets in contrast with other imidazoline/guanidine insulin releasing agents, such as efaroxan, which possess high affinity α_2-adrenoceptor antagonism. A good correlation between the concentration of BTS 67 582 which increased insulin release from islets

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and the plasma concentrations modulating plasma glucose in rats, supports the hypothesis that the glucose lowering activity of the compound is predominantly due to the stimulation of insulin release.

2. Materials and methods

2.1. Isolation of pancreatic islets

Pancreatic islets were isolated from fed male Charles River Wistar rats (175–225 g) using a method based on the ductal injection of collagenase as used by previous workers (Lacy and Kostianovsky, 1967). Oxygenated (95% O_2/5% CO_2) Gey and Gey buffer (Gey and Gey, 1936) was used throughout with the following constituents (in mM): NaCl (111), $NaHCO_3$ (27), KCl (4.96), $CaCl_2$ (2.0), $MgCl_2 \cdot 6H_2O$ (0.98), $MgSO_4 \cdot 7H_2O$ (0.28), Na_2HPO_4 (0.63), KH_2PO_4 (0.22), 1 mg/ml bovine serum albumin (Sigma, Fraction V, RIA grade) and 4 mM glucose unless otherwise described. Rats were killed by cervical dislocation and exsanguinated by cutting the abdominal aorta. The branch of the bile duct leading to the liver and the duodenal end of the duct in the pancreas were clamped and the pancreas distended by the injection of 10 ml of ice-cold 0.9 mg/ml collagenase (Sigma, Type XI) solution into the bile duct using a syringe and 23 gauge needle. The pancreas was then removed and incubated statically for 12 min at 37°C. Following the incubation, 10 ml of cold buffer was added and the suspension shaken vigorously by hand for 1 min. The islets were allowed to settle for 5 min on ice and washed three times using ice-cold buffer. Islets from 3 rats were picked and pooled and a final selection of islets then transferred to the perifusion apparatus.

2.2. Perifusion of pancreatic islets

Batches of 20 islets were placed into disposable syringe filters (Gelman Acrodisc 0.8 μm) and perifused at 37°C with Gey and Gey buffer at 1 ml/min. Each perfusion chamber was fed from two flasks (wash and treatment) selected via a three way tap placed immediately before the filter. Flasks, tubing and filters were immersed in a water bath. Flasks were continuously surface gassed with 95% O_2/95% CO_2 at 3 l/min. Buffer was pulled through the system using a peristaltic pump (Gilson Minipuls 2 fitted with 1 mm tubing) placed outside the water bath and samples collected at 2 min intervals unless otherwise stated. The total dead volume of the system from the reservoir to the filter was 1.2 ml and the volume from the filter to the collection tube (including peristaltic pump) was 1 ml. A total of 6 perifusion systems were run simultaneously for each experiment which consisted of 40 min pre-equilibration (of which fractions from the last ten min were collected) followed by one hour with the relevant treatment unless otherwise stated. Fractions were stored at −75°C until required for assay. Insulin content of the fractions was measured using commercial radioimmunoassay kits (Linco, rat insulin RI-13K). Insulin release from the islets was expressed in units of pg insulin released/islet/min.

2.3. Effect of BTS 67 582 on fasted rats

Normal male Wistar rats (Harlan UK, 175–210 g) were starved overnight, but allowed water ad libitum. Blood was sampled prior to and 1.5 hours after dosing with BTS 67 582. Preliminary studies had indicated that maximal blood levels and glucose lowering activity of BTS 67 582were achieved 1 to 2 h after oral dosing. Blood sampling was by tail vein venipuncture with a 23 g butterfly needle and facilitated by placing the rats into a heated cabinet at 37°C for 15 min prior to blood sampling. Blood was collected into heparinised tubes and centrifuged at 5000 g for 3 min. Plasma glucose concentration was immediately measured in duplicate using a glucose oxidase procedure (Analox GM6 blood glucose meter). Plasma samples for insulin assay were stored at −75°C until assayed using a commercial rat RIA (Biotrak Amersham, RPA 547). Plasma samples for the determination of BTS 67 582 concentration were stored at −75°C until required.

Plasma samples (100 μl) for determination of BTS 67 582 concentration together with an internal standard (1-butyl-2-(2-morpholinophenyl) iminoimidazolidine fumarate) were passed onto conditioned 1 ml C18 Bond-Elut (Varian) solid-phase extraction cartridges and washed with 1 ml of 10% methanol in water. BTS 67 582 was then eluted with 1 ml of 1% acetic acid in methanol and the eluate dried with a stream of nitrogen at 37°C. The residue was then dissolved in 100 μl of High Performance Liquid Chromatography (HPLC) mobile phase. The recovery of BTS 67 582 from rat plasma with respect to an external standard was found to be 71%.

Aliquots of 85 μl from the solid phase extraction were then assayed for BTS 67 582 concentration using HPLC with a Spherisorb or Exsil nitrile column (200 or 250 × 4.6 mm), fitted with a 10 mm × 2 mm pellicular nitrile guard column, at a temperature of 40°C. Mobile phase (acetonitrile: pentane sulphonic acid (5 mM with 0.5% v/v acetic acid), 70:30) at a flow rate of 1.6 ml/min was used. Quantitation of BTS 67 582 was by a uv monitor at 225 nm and peak area measured using an electronic integrator. The retention times of BTS 67 582 and internal standard under these conditions were approximately 6.5 and 7.6 min respectively.

2.4. Drugs

BTS 67 582 and the internal standard for HPLC analyses were synthesised by Medicinal Chemistry, Knoll Pharmaceuticals. All other chemicals and drugs were from

Sigma-Aldrich (Poole, Dorset) or RBI International (Natick, MA). For oral dosing (1–300 mg/kg) BTS 67 582 was dissolved in 0.25% cellosize in distilled water and dosed by gavage at 10 ml/kg. For all other studies, drugs were dissolved in the appropriate assay buffer.

2.5. Statistical analysis of results

Insulin release from perifused pancreatic islets is presented as the mean of at least 3 separate experiments and graphs shown as the mean ± SEM. Mean insulin release was determined for the 60 min following drug addition, using a square root transformation to account for the data being positively skewed. A sigmoidal concentration-response curve was fitted to the transformed data by non linear regression (SAS, Cary, NC) to determine EC_{50} values (defined as the concentration that gave a response halfway between the control response and the estimated maximum). All EC_{50} values are quoted along with 95% confidence intervals.

The ED_{50} and EC_{50} values for the studies in fasted rats were defined as the dose or concentration that gave a plasma glucose level half-way between that for control and the estimated maximum response. Geometric means were adjusted for differences between treatments at baseline and data analysed by fitting a sigmoidal dose-response curve. EC_{50} and ED_{50} values are quoted along with 95% confidence intervals.

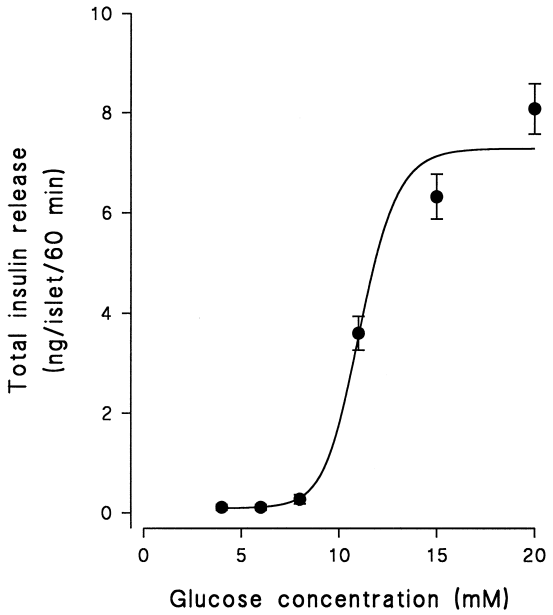

Fig. 1. Effect of glucose on total insulin release from perifused rat islets. Islets were perifused for an initial period of 40 min at 4 mM glucose to allow equilibration prior to the introduction of the appropriate glucose concentration. Each point represents the total insulin release for the 60 min experiment following treatment and is presented as a mean of three independent experiments ± SEM.

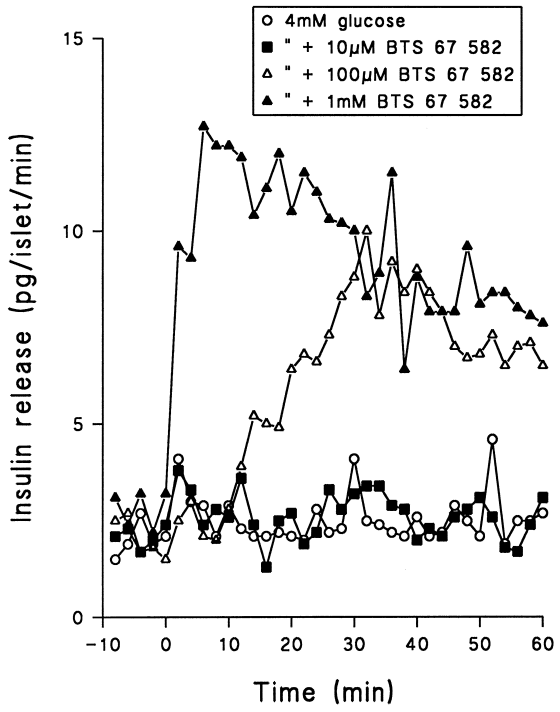

Fig. 2. Effect of BTS 67 582 on insulin release from perifused rat islets at 4 mM glucose. Islets were perifused for an initial period of 40 min at 4 mM glucose to allow equilibration and fractions taken for ten min before BTS 67 582 was introduced in the same buffer. Each point is the mean of three independent experiments. SEM's were from 50% to 150% of the mean and not shown for the sake of clarity.

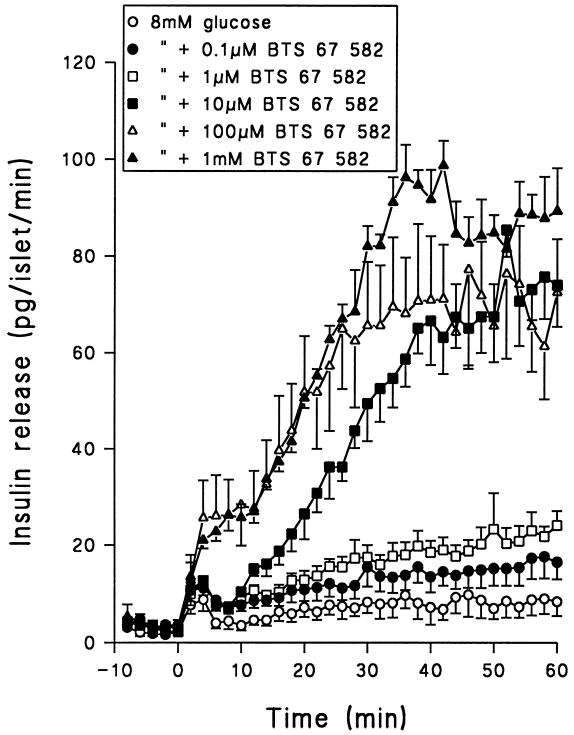

Fig. 3. Effect of BTS 67 582 on insulin release at 8 mM glucose from perifused rat islets pre-equilibrated with 4 mM glucose. Islets were perifused for an initial period of 40 min at 4 mM glucose to allow equilibration and fractions taken for ten min before BTS 67 582 was introduced in 8 mM glucose. Each point is the mean of three independent experiments and is shown as ± SEM.

3. Results

3.1. Effect of BTS 67 582 on insulin release from perifused pancreatic islets

Control experiments indicated that the perifused pancreatic islets responded to increasing glucose concentrations with a large increase in insulin secretion. The EC_{50} value for glucose stimulation of total insulin release was 11.1 (10.6, 11.6) mM and total insulin release over the 60 min of the experiment was increased approximately 100 fold at 20 mM glucose relative to 4 mM glucose (Fig. 1). There was no increase of insulin secretion, relative to the 4 mM control, with 6 mM glucose. Total insulin release over the 60 min of the experiment was, however, increased 2.3 fold, relative to 4 mM glucose, when islets were perifused with 8 mM glucose.

Perifusion at the substimulatory glucose concentration of 4 mM led to an elevation of insulin release with BTS 67 582 at only 100 μM and 1 mM BTS 67 582 (Fig. 2). The increase in insulin secretion with 1 mM BTS 67 582 at 4 mM glucose represented less than 10% of the maximal glucose-dependent increase. BTS 67 582 at concentrations of up to 1 mM had no effect on insulin release when islets were perifused with 15 mM glucose (data not shown).

In contrast, at the submaximal stimulatory concentration of 8 mM glucose there were clear effects of BTS 67 582 on insulin release which were assessed in two different ways. Firstly pancreatic islets were pre-equilibrated with 4 mM glucose and incubated in 8 mM glucose with varying concentrations of BTS 67 582 (Fig. 3). This series of experiments was then repeated, but with islets pre-equilibrated with 8 mM glucose, again incubated at 8 mM glucose with varying concentrations of BTS 67 582 (Fig. 4). The EC_{50} value for the effect of BTS 67 582 on total insulin secretion at 8 mM glucose was determined to be 7.7 (2.8, 21.4) μM when the islets were pre-equilibrated with 4 mM glucose (Fig. 3) and 6.3 (1.4, 27.9) μM when the islets were pre-equilibrated with 8 mM glucose (Fig. 4). Pre-equilibration with 8 mM glucose clearly did not affect the observed potency of BTS 67 582 but did reduce the total increase in insulin release relative to control by a considerable amount. Islets pre-equilibrated with 8 mM glucose did not recover as well from the isolation procedure as indicated by a considerably higher basal insulin release and a reduced magnitude of glucose response. The reduced secretory response of these islets thus reduced the apparent effects of drugs on the magnitude of insulin release. However, prior equilibration at 8 mM glucose showed more clearly the effects of BTS 67 582 on first and second phase insulin release. BTS 67 582 appears to

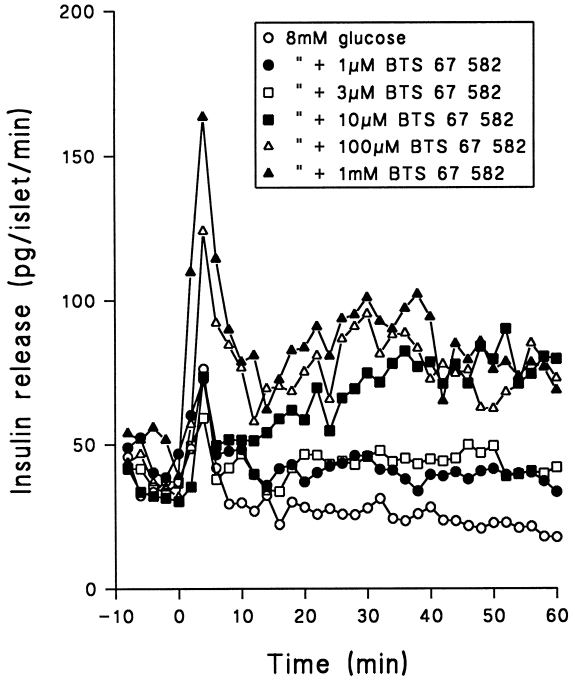

Fig. 4. Effect of BTS 67 582 on insulin release at 8 mM glucose from rat islets pre-equilibrated with 8 mM glucose. Islets were perifused for an initial period of 40 min at 8 mM glucose to allow equilibration and fractions were taken for ten min before BTS 67 582 was introduced in the same buffer. Each point is the mean of three independent experiments. SEM's were from 70% to 130% of the mean and not shown for the sake of clarity.

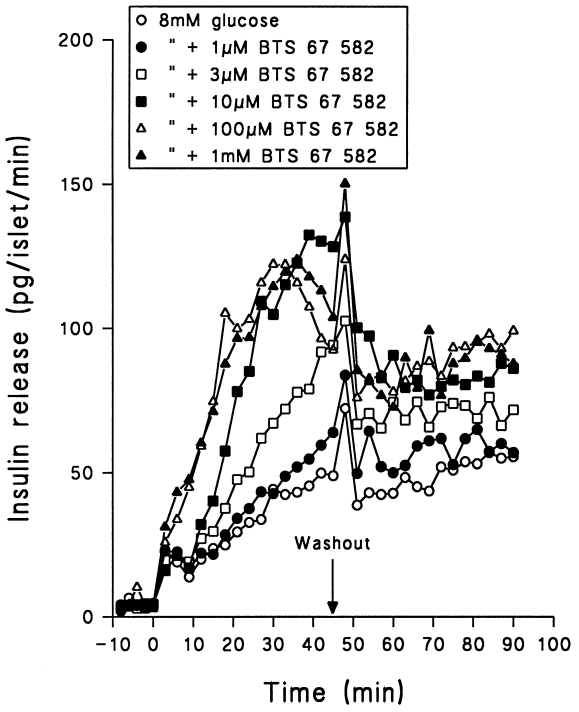

Fig. 5. Reversibility of the effect of BTS 67 582 on insulin release at 8 mM glucose from rat islets pre-equilibrated with 4 mM glucose. Islets were perifused for an initial period of 40 min at 4 mM glucose to allow equilibration and fractions taken for ten min before BTS 67 582 was introduced in 8 mM glucose. Fractions were taken at 3 min intervals for a total of 45 min and then BTS 67 582 was withdrawn and islets perifused for a further 45 min with 8 mM glucose. Each point is the mean of three independent experiments. SEM's were from 50% to 150% of the mean and not shown for the sake of clarity.

stimulate the second phase insulin release more potently than first phase as a clear stimulation of first phase insulin release is only seen for the highest two concentrations (Fig. 4). There is also, however, a transient increase in insulin secretion visible at this point for the control islets. This effect appears to be due to pressure fluctuations on the islets when perifusing solutions are switched. This effect, whilst slight, is most pronounced for this series of experiments and may have masked minor effects of the lower concentrations of BTS 67 582 on the first phase of insulin secretion.

The effects of BTS 67 582 on insulin release at 8 mM glucose were, for the most part, rapidly reversed following a 45 min washout period (Fig. 5). For this series of experiments there was a much greater basal response of the islets to 8 mM glucose which reduced the magnitude of the effect of BTS 67 582 on insulin secretion. Following removal of drug, the majority of the BTS 67 582 induced increase of insulin secretion rapidly disappeared but there was a considerable degree of experiment to experiment variability in the magnitude of this drop. It does seem, however, that there may be a residual effect of BTS 67 582 on insulin secretion that persists following a 45 min washout period, though this does not seem to be associated with any acute toxicity. Further work would be required to clarify the issue of whether there is a real long term effect of BTS 67 582 on insulin secretion.

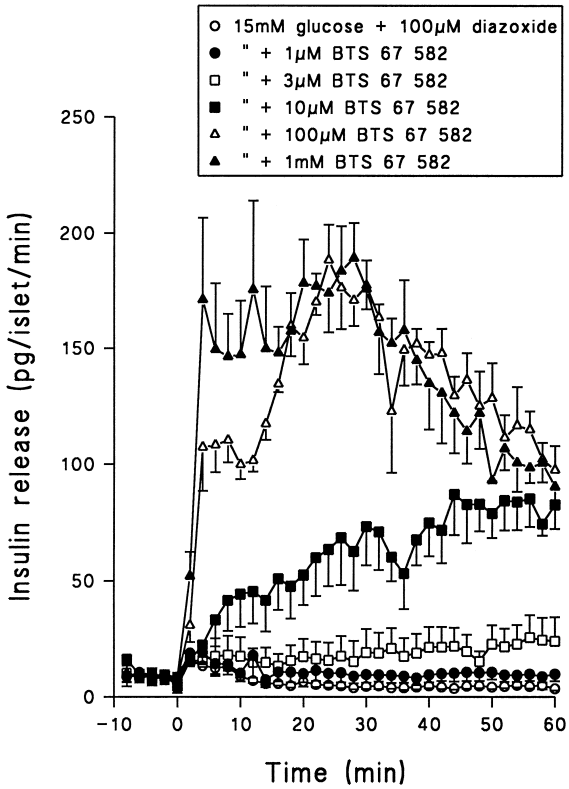

Fig. 6. Effect of BTS 67 582 on insulin release from rat islets pre-equilibrated with 15 mM glucose and 100 μM diazoxide. Islets were perifused for an initial period of 40 min at 15 mM glucose and 100 μM diazoxide to allow equilibration and fractions taken for ten min before BTS 67 582 was introduced in the same buffer. Each point is the mean of three independent experiments and is shown as $\pm$SEM.

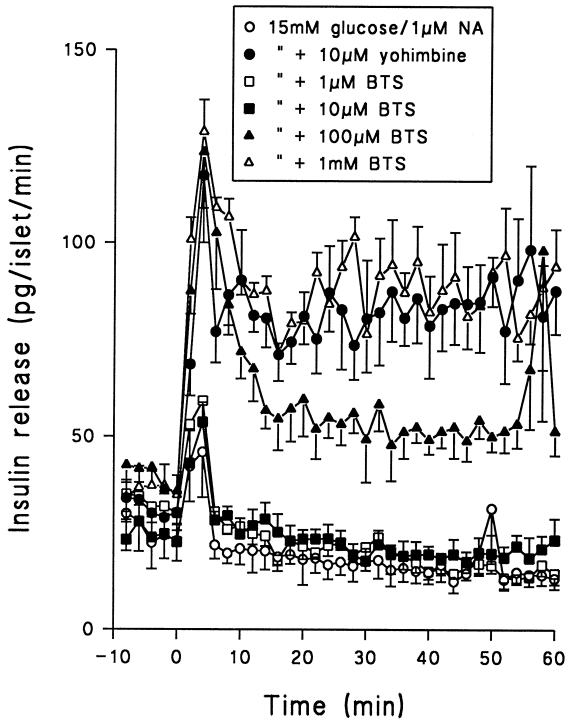

Fig. 7. Effect of BTS 67 582 on insulin release from rat islets pre-equilibrated with 15 mM glucose and 1 μM noradrenaline. Islets were perifused for an initial period of 40 min at 15 mM glucose and 1 μM noradrenaline to allow equilibration and fractions were taken for ten min before BTS 67 582 was introduced in the same buffer. Each point is the mean of three independent experiments and is shown as $\pm$SEM.

Total insulin release, over 60 min, at 8 mM glucose with 100 μM BTS 67 582 was reduced from 3.96 ± 0.16 to 1.84 ± 0.11 ng/islet/60 min in the presence of the L-type Ca^{2+} channel blocker ($\pm$)-verapamil at 10 μM (mean $\pm$ SEM, 3 experiments). Baseline insulin secretion under these conditions with 8 mM glucose was 2.36 ± 0.63 ng/islet/60 min. This experiment indicated that verapamil could completely suppress the stimulation of insulin release at 8 mM glucose by 100 μM BTS 67 582 indicating that Ca^{2+} entry was required for this effect.

Diazoxide inhibits glucose-dependent insulin release by selectively opening the ATP-dependent K^+ channel in pancreatic β-cells (reviewed by Dunne et al., 1996). Diazoxide (100 μM) nearly completely suppressed the effect of 15 mM glucose on insulin secretion by pancreatic islets. BTS 67 582 was able to reverse the inhibitory effect of 100 μM diazoxide on islets pre-equilibrated with diazoxide and 15 mM glucose (Fig. 6) suggesting that the compound is acting as a functional ATP-dependent K^+ channel blocker in this tissue; the EC_{50} value for this effect on total insulin secretion was 13.2 (8.9, 19.6) μM. Total insulin release from these islets at the highest dose of BTS 67 582 was similar to control experiments with 15

mM glucose only (Fig. 1) indicating that the compound was able to fully inhibit the effect of diazoxide on insulin release.

Noradrenaline inhibits glucose-dependent insulin release by activating the α_2-adrenoceptors in pancreatic β-cells (Morgan and Montague, 1985). This study confirmed these results in that the addition of 1 μM noradrenaline largely prevented glucose dependent insulin release from perifused islets and that yohimbine at 10 μM could completely reverse this inhibition. BTS 67 582 reversed this inhibition of insulin release only at high concentrations (Fig. 7) and an EC_{50} of 83 (67, 102) μM was obtained. These data suggest that BTS 67 582 can act as a weak α_2-adrenoceptor antagonist. Total insulin release from these islets at the highest dose of BTS 67 582 was similar to control experiments with 15 mM glucose only (Fig. 1) indicating that the compound was able to fully inhibit the effect of noradrenaline on insulin release.

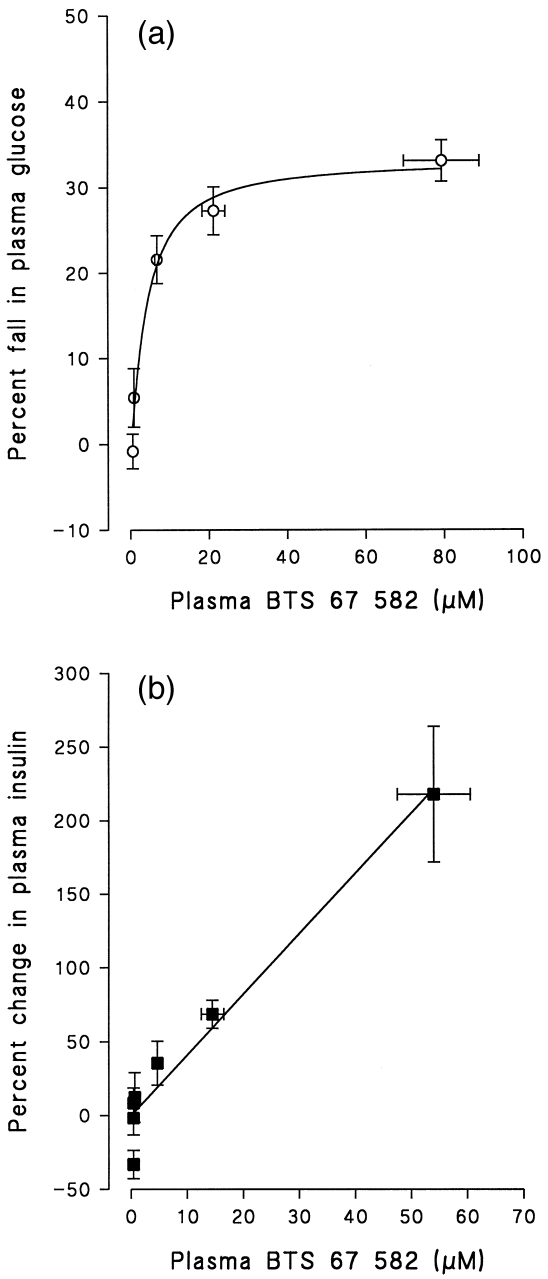

Fig. 8. Relationship between plasma concentration of orally dosed BTS 67 582 and effect on plasma glucose (a) and insulin (b) concentrations in normal fasted rats. Plasma was sampled prior to and 1.5 hours after dosing with BTS 67 582 (1–300 mg/kg) and assayed for glucose, insulin and BTS 67 582 ($n = 6$–12). An EC_{50} value of 3.9 (1.9, 7.9) μM for the effect of BTS 67 582 on plasma glucose was obtained whilst a linear regression line ($r^2 = 0.958$) was fitted to the graph showing the effect on insulin.

Efaroxan, an α_2-adrenoceptor antagonist and imidazoline insulin releasing agent, was able to potently and completely reverse the noradrenaline mediated inhibition of insulin release (3 experiments, data not shown) with an EC_{50} of 122 (47, 314) nM. The potency of efaroxan in reversing the noradrenaline inhibition of insulin release in this study is similar to previously reported data (Chan and Morgan, 1990). In contrast, the sulphonylurea insulin releasing agent, glibenclamide, at 1 μM had no effect on insulin secretion from islets in the presence of noradrenaline (data not shown).

3.2. Effect of BTS 67 582 on fasted rats

Fasted rats had a mean plasma glucose concentration of 5.77 $\pm$ 0.09 mM glucose (mean $\pm$ SEM, $n = 12$). BTS 67 582 (1–300 mg/kg) reduced plasma glucose by a maximum of 30.9 $\pm$ 2.4% (300 mg/kg, $n = 6$), 1.5 hours after dosing, with an ED_{50} value of 17.0 (10.5, 27.7) mg/kg. There was a corresponding 10.8% drop in blood glucose for vehicle dosed animals and the 300, 100 and 30 mg/kg doses were significantly different from this value (Williams' test $P < 0.001$). The threshold for this effect was approximately 3–10 mg/kg and was maximal at approximately 100 mg/kg. Fig. 8a shows the relationship between plasma concentrations of BTS 67 582 and percentage fall in plasma glucose. A curvilinear response was obtained with an associated EC_{50} value of 3.9 (1.9, 7.9) μM. There was an approximately linear relationship between plasma insulin concentration and plasma BTS 67 582 concentration ($r^2 = 0.958$, Fig. 8b). Plasma protein binding ofBTS 67 582 was determined to be less than 10% (data not shown) and so would have affected these results by only a minor amount.

4. Discussion

BTS 67 582 acts as an insulin releasing agent in perifused rat pancreatic islets. It appears to act by potentiating the effect of glucose on insulin secretion in pancreatic islets and has minimal effects at non stimulatory and near maximal stimulatory concentrations of glucose. The majority of the BTS 67 582 promoted increment in insulin

secretion disappeared rapidly on removal of the drug though there was a minor component that appeared to remain for at least 45 min. The ability of BTS 67 582 to reverse the inhibitory effect of diazoxide on insulin release with a similar potency to its effect on direct insulin release suggests that insulin release is mediated by functional K^+ channel blockade at the ATP-dependent K^+ channel confirming preliminary data on patch-clamped β-cell membranes (Jones et al., 1996). We have also shown in this study that the potency of BTS 67 582 to lower blood glucose in vivo in fasted rats (EC_{50} of 3.9 μM) is very similar to its potency in vitro in pancreatic islets (EC_{50} of 7.7 and 6.3 μM). It has previously been shown that BTS 67 582 lowered plasma glucose and raised plasma insulin concentration in a streptozotocin-induced model of diabetes whereas glibenclamide was inactive (Jones et al., 1997). This work indicated that BTS 67 582 possessed a different mechanism of action to that of the sulphonylureas but that this effect was still mediated by insulin release. In human volunteers plasma concentrations of BTS 67 582 between 0.1 μM and 7 μM produced significant lowering in plasma glucose concentrations (Byrom et al., 1994). The similarity of the potency of BTS 67 582 to promote insulin secretion in pancreatic islets with its glucose lowering potency in both rats and man strongly suggests that BTS 67 582 lowers blood glucose in vivo by its activity to directly stimulate insulin secretion.

The presence of the guanidine group in BTS 67 582 leads to a consideration of an interaction at the as yet uncharacterised imidazoline/guanidine receptor mediating insulin secretion and whether this might account for the activity of the compound on insulin release. Recent work has shown that an imidazoline receptor appears to mediate insulin release from pancreatic islets (for reviews see: Chan, 1993; Morgan et al., 1995). Interaction at this putative imidazoline binding site appears to promote insulin secretion via direct inhibition of the ATP-dependent K^+ channel, at a site different to that of the sulphonylureas (reviewed by Dunne et al., 1996). The ability of BTS 67 582 to inhibit the ATP-dependent K^+ channel in patch clamped β-cell membranes is consistent with this hypothesis (Jones et al., 1996). The same study also showed that trypsin treatment of the patch resulted in a loss of sensitivity of the ATP-dependent K^+ channel to sulphonylureas, but not to BTS 67 582. The resistance of the ATP-dependent K^+ channel to trypsin treatment in patch clamped β-cells has also been shown for imidazoline releasing agents (Dunne et al., 1996). It seems possible that at least part of the effect of BTS 67 582 on insulin secretion is mediated by an interaction at the putative insulin releasing islet imidazoline/guanidine receptor.

Many of the imidazoline/guanidine containing insulin releasing agents are also α_2-adrenoceptor antagonists. Indeed it was thought for many years that these agents caused insulin release in vivo by antagonising the tonic inhibitory effect of noradrenaline at α_2-adrenoceptors on insulin release. Recent work has however shown that a separate receptor must also be involved (for reviews see: Chan, 1993; Morgan et al., 1995). The insulin releasing imidazolines, such as efaroxan, tend to be potent α_2-adrenoceptor antagonists, as shown by the inhibition of the noradrenaline effect on insulin release, but relatively much weaker direct insulin releasing agents in vitro (Chan and Morgan, 1990). Similar results have also been shown recently for the imidazoline containing insulin releasing agent SL 84.0418 (Jonas et al., 1994). In contrast to efaroxan and SL 84.0418, BTS 67 582 in this study, had only weak activity in blocking the inhibition of insulin release by noradrenaline relative to its potency at directly stimulating insulin release, thus indicating that the compound does not possess significant antagonistic activity at the islet α_2-adrenoceptor.

In summary, BTS 67 582 acts as an insulin releasing agent in pancreatic islets in a manner consistent with an interaction at the ATP-dependent K^+ channel. The potency of this effect in vitro is similar to the potency of the glucose lowering effect of BTS 67 582 in fasted rats. The lack of any binding to the sulphonylurea receptor suggests that BTS 67 582 may interact at the putative islet imidazoline/guanidine receptor thought to be involved in insulin release and which also appears to regulate activity of the ATP-dependent K^+ channel. Recent work showing that BTS 67 582 has very similar electrophysiological effects to efaroxan in patch clamped β-cell membranes appears to support this hypothesis (Jones et al., 1996). As all insulin releasing agents currently used clinically for NIDDM appear to work by binding to the sulphonylurea receptor associated with the ATP-dependent K^+ channel (for reviews see: Ashcroft and Ashcroft, 1992; Edwards and Weston, 1993), there may be clinical benefits from insulin releasing agents interacting at other sites on the ATP-dependent K^+ channel.

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