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Evaluation of BTS 67 582, a novel antidiabetic agent, in normal and diabetic rats

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1 The effect of BTS 67 582, a novel antidiabetic agent, has been evaluated on plasma glucose and plasma insulin in normal and streptozotocin-induced diabetic rats.

2 BTS 67 582 (3 to 300 mg kg^{-1}, p.o.) caused a dose- and time- dependent reduction in plasma glucose and an increase in plasma insulin in both fasted and glucose-loaded normal rats. The ED_{50} for the glucose lowering effect of BTS 67 582 in fasted rats was 37.6, 18.4 and 18.5 mg kg^{-1} at 1, 2 and 4 h after administration respectively.

3 In streptozotocin-induced (50 mg kg^{-1}, i.v.) diabetic rats, BTS 67 582 (37–147 mg kg^{-1}, p.o.) caused significant reductions of plasma glucose following a glucose load, whereas glibenclamide (100 mg kg^{-1}, p.o.) was ineffective. BTS 67 582 significantly increased plasma insulin compared to controls whereas glibenclamide did not.

4 BTS 67 582 did not displace [^{3}H]-glibenclamide from its binding sites in rat brain, guinea-pig ventricle or the HIT-T15 insulinoma β-cell line. BTS 67 582 does not therefore appear to modulate its action via an effect on the 'sulphonylurea' receptor.

5 In fasted rats, the glucose lowering effect of BTS 67 582 (100 mg kg^{-1} p.o.) and glibenclamide (1 mg kg^{-1}, p.o.) were antagonized by diazoxide (30 mg kg^{-1}, i.p.). In addition BTS 67 582, like glibenclamide, caused a dose-dependent rightward shift of cromakalim-induced relaxation of noradrenaline precontracted rat aortic strips, suggesting the involvement of K_{ATP} channels.

6 In summary, BTS 67 582 produces a blood glucose-lowering effect in normal and streptozotocin-induced diabetic rats associated with increased insulin concentrations. This effect appears to be due to a blockade of ATP-sensitive potassium channel activity via a different binding site to that of glibenclamide.

Keywords: BTS 67 582; plasma glucose; plasma insulin; streptozotocin-induced diabetes

Introduction

The sulphonylurea class of compounds has been in widespread use for the treatment of non insulin dependent diabetes mellitus (NIDDM) for many years. These compounds all display similar chemical structural characteristics and appear to exert their hypoglycaemic effect via an action on the 'sulphonylurea' receptor, but differ clinically in their pharmacodynamic and pharmacokinetic parameters. Sulphonylureas block the pancreatic islet β-cell adenosine 5′-triphosphate (ATP)-dependent potassium channel (K_{ATP}) channel, causing depolarisation of the β-cell membrane, calcium influx and insulin secretion (Ashcroft & Ashcroft, 1992; Edwards & Weston, 1993).

BTS 67 582 (1,1-dimethyl-2-(2-morpholinophenyl)guanidine fumarate (Figure 1)), is a novel glucose lowering agent, currently under clinical investigation for the treatment of NIDDM. It is structurally unrelated to the sulphonylurea class of K_{ATP} channel blockers. The glucose lowering and insulin stimulating characteristics of BTS 67 582 in normal and streptozotocin-induced diabetic rats have been demonstrated and compared with tolbutamide and glibenclamide, examples of first and second generation sulphonylureas, respectively. In this study, evidence is provided to show that BTS 67 582 does not act through the same binding site as glibenclamide. However, functional potassium channel blockade has been demonstrated and BTS 67 582 may therefore represent a novel class of potassium channel blocker with potential value in the treatment of NIDDM. Preliminary accounts of part of this work have been presented previously (Kaul *et al*., 1995a,b).

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Methods

Studies on plasma glucose and insulin

Normal male Wistar rats (130–240 g) were deprived of food overnight in wire mesh cages but allowed water *ad libitum*. Blood samples (approx 0.3 ml) were taken by venepuncture from the tail vein into heparinized tubes (Sarstedt Microvette CB1000S) and centrifuged at $5000 \times g$ for 3 min. Rats were warmed in a heated cabinet at 37°C for 15 min before venepuncture. Plasma samples (10 μl) were immediately analysed for glucose content by use of a glucose oxidase procedure (Analox GM6 blood glucose meter). Plasma samples (120 μl) for insulin determinations were frozen (−78°C) until assayed by RIA with rat insulin standards (BioTrak Amersham, RPA 547). In glucose loading experiments, rats received D-glucose 800 mg kg^{-1}, s.c., immediately before oral administration of test compounds. Drugs were dissolved, or made up as a suspension, in 0.25% cellosize and administered orally at 10 ml kg^{-1}. Vehicle control animals received 0.25% cellosize only.

Glucose absorption from the intestine

Normal Wistar fasted rats (180–220 g) received BTS 67 582 (100 mg kg^{-1}) two hours before glucose loading (2 g kg^{-1} in 20% w/v solution) directly into the jejunum. Thirty minutes before the glucose load, pentobarbitone anaesthesia was induced (approx 45 mg kg^{-1}), and the hepatic portal vein cannulated without obstructing the vessel. To assess the initial rate of glucose absorption, blood samples for plasma glucose determination were taken from the hepatic portal vein at intervals over 15 min immediately after glucose loading.

Diazoxide induced hyperglycaemia

Normal Wistar fasted rats (180–220 g) were dosed with diazoxide to suppress insulin release at the level of the K_{ATP} channel of the islet β-cell. Diazoxide was given at an initial dose of 30 mg kg^{-1}, i.p., 30 min before administration of BTS 67 582 or glibenclamide. Supplementary doses of diazoxide were given (15 mg kg^{-1}, i.p.) during the experiment every 15 min. Blood samples were taken at intervals up to 3 h after administration of test substances.

Rat aortic strips

Normal male Wistar rats (180–240 g) were killed by cervical dislocation and the descending thoracic aorta was rapidly removed and placed in ice-cold Krebs Henseleit solution of the following composition (mM: NaCl 118.4, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0 and D-glucose 10.1). The aorta was cut into a spiral strip, one end being attached to the base of a 15 ml organ bath, the other to an isotonic transducer (Harvard, tension adjusted to 1 g), and contractions and relaxations recorded continuously on a polygraph (Grass model 79). The tissue was bathed in Krebs Henseleit solution at 37°C (pH 7.4) and continually gassed with 95%O_2/5%CO_2. The tissue was repeatedly washed and allowed to stabilize over 0.5–1 h. Noradrenaline (10^{-8} M, previously determined to cause an approximate 80% of maximal contraction) was added to the organ bath and the contraction allowed to develop and stabilize. Cromakalim (10^{-9} M to 10^{-5} M, as appropriate) was then added in a cumulative manner to the organ bath in half log increments to relax the tissue. Additional doses were added when a plateau to the previous dose had been observed. When maximal relaxation had been achieved, the tissues were washed repeatedly over approximately 45 min to restore baseline values. The procedure was then repeated, the aorta being contracted with noradrenaline and relaxed with cromakalim, in the presence of either BTS 67 582 (10^{-6}, 10^{-5} and 10^{-4} M) or glibenclamide (10^{-7}, 10^{-6}, and 10^{-5} M). Stock solutions of drugs were made up in dimethylsulphoxide (DMSO) and then diluted in assay buffer (maximal DMSO concentration in organ bath was >0.1% which had no effect on contractility). BTS 67 582 was

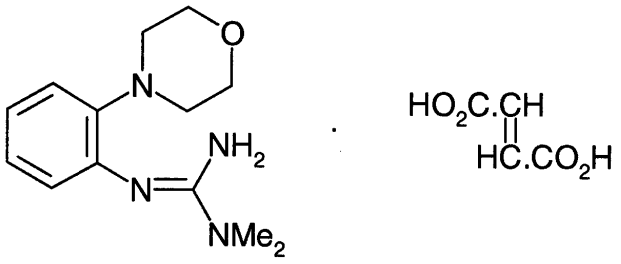

Figure 1 Structure of BTS 67 582.

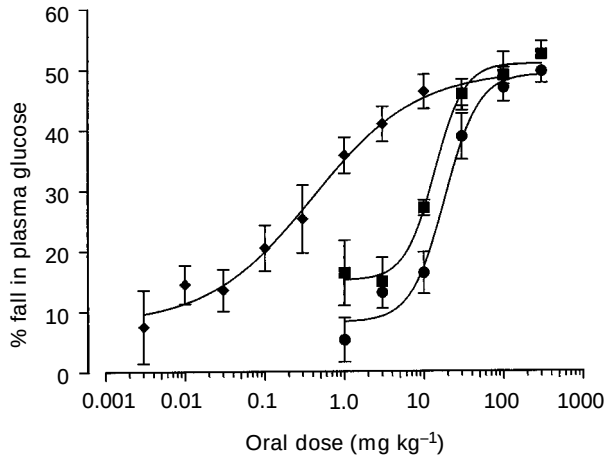

Figure 3 Relationship betwen oral dose and percentage reduction in plasma glucose in normal fasted rats 2 h after dosing with BTS 67 582 (●) glibenclamide (◆) and tolbutamide (■), $n=8-12$. ED_{50} values were 18.4, 0.4 and 13.7 mg kg^{-1}, respectively. Average plasma glucose fall in vehicle-dosed rats was 8.3%. Vertical lines show s.e.mean.

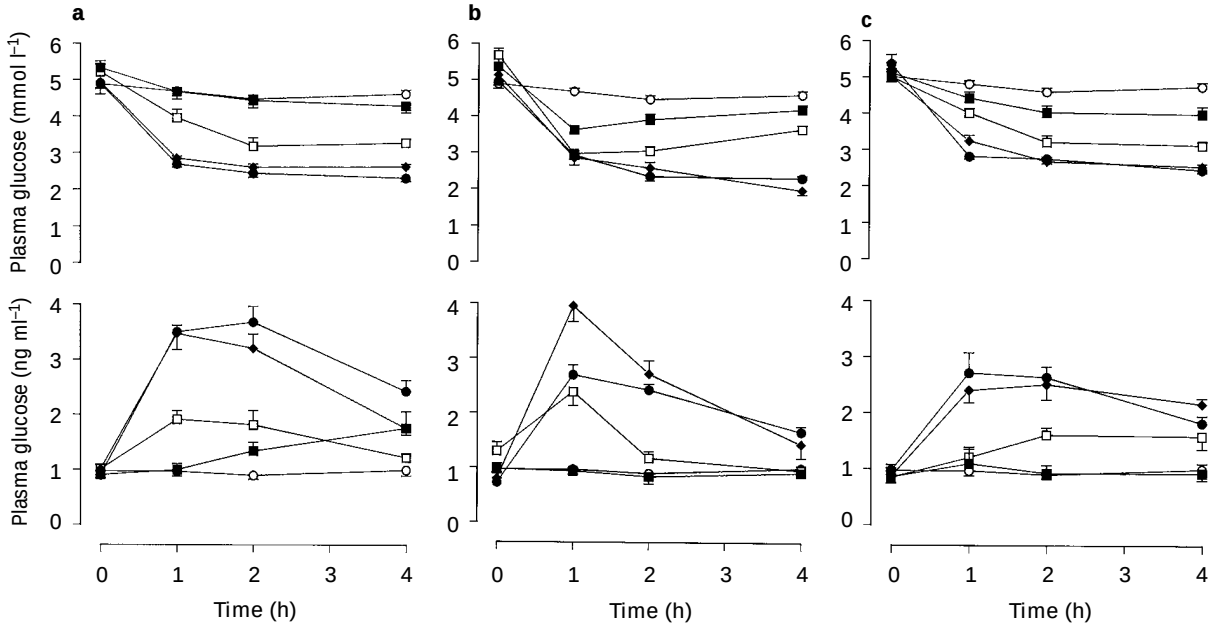

Figure 2 Effect of (a) BTS 67 582 300 (●), 100 (◆), 30 (□) and 10 (■) mg kg^{-1}, (b) tolbutamide 300 (●), 100 (◆), 30 (□) and 10 (■) mg kg^{-1}; and (c) glibenclamide 100 (●), 10 (◆), 1.0 (□) and 0.1 (■) mg kg^{-1} on plasma glucose and insulin in normal fasted rats. $n=6-12$ except vehicle (○) where $n=24-28$. All compounds were dosed orally at 0 hour. Some doses were omitted for clarity. Vertical lines show s.e.mean.

dissolved in assay buffer. The effects of cromakalim on relaxation were expressed as a percentage of the noradrenaline-induced contraction.

Streptozotocin-induced diabetes

Diabetes was induced in female Sprague Dawley rats (160–230 g body weight) by the injection of streptozotocin (STZ, Sigma, 50 mg kg^{-1}, i.v. dissolved in 0.05 M citrate buffer, pH 4.5) via the tail vein. Animals were deprived of food (but allowed water *ad libitum*), overnight before and for 2–3 h following, the streptozotocin injection. After 10–14 days the animals were fasted overnight and given an oral glucose load (D-glucose, 800 mg kg^{-1}) 30 min after a high dose of glibenclamide (100 mg kg^{-1}). Only those animals which displayed plasma glucose concentrations greater than 15 mM 3 h after glibenclamide administration were selected for further study. These animals were defined as resistant to the effects of glibenclamide. These STZ rats were considered to be a model of moderate to severe NIDDM since they were hyperglycaemic and possessed low concentrations of circulating insulin. They did not require injections of insulin for survival and therefore were not a model of IDDM.

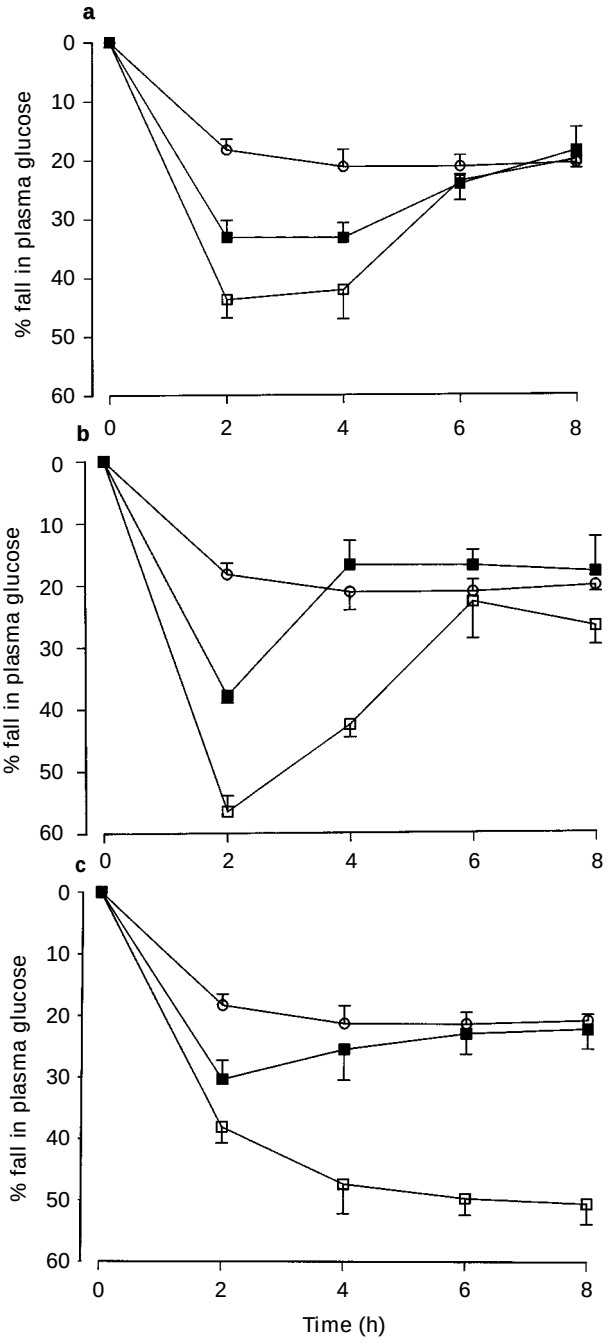

Figure 4 Effect of vehicle (○) and (a) BTS 67 582 at 30 (■) and 100 (□) mg kg^{-1}, (b) tolbutamide at 30 (■) and 100 (□) mg kg^{-1}, and (c) glibenclamide at 0.5 (■) and 10 (□) mg kg^{-1}, on percentage reduction of plasma glucose in normal fasted rats ($n=4$). Vertical lines show s.e.mean.

Urinary excretion studies

The potential for BTS 67 582 to alter urinary glucose excretion was evaluated in glucose loaded STZ rats (as described above). Fasted STZ rats were placed in metabolism cages (2 per cage). Urine was collected from 0 to 3.5 h and from 3.5 to 6.5 h after glucose administration and the volume noted. The cage was then rinsed with distilled water, this being added to the urine. Glucose content was measured as described above. Samples for plasma glucose determination were taken before and at 1 and 3.5 h after administration of BTS 67 582 293 mg kg^{-1}, p.o.

[^{3}H]-glibenclamide binding studies

Brains from male Sprague Dawley rats (200–250 g) were homogenized in two volumes of ice-cold 50 mM Tris-Cl buffer, pH 7.4, with a Polytron homogenizer, setting No 4, with a PT7.5 head with two ten second bursts. Total membranes were harvested by centrifugation at 40,000 *g* for one hour. The pellet was washed once and finally resuspended in 50 mM Tris-Cl buffer for storage under liquid nitrogen until required. Heart membranes from male Dunkin-Hartley guinea-pigs (300–400 g) were prepared in the same way as the rat brain membranes except that 50 mM Tris-Cl buffer, pH 7.7 was used. HIT-T15 hamster insulinoma β-cells were grown as previously described (Ashcroft *et al*., 1986) and stored under liquid nitrogen until required. HIT-T15 β-cells were homogenized (Polytron PT7.5) in 20 volumes of 50 mM Tris-Cl buffer, pH 7.7 and total membranes prepared as above. Protein concentrations were assayed by a modification of the Lowry procedure (Markwell *et al*., 1978).

Incubations were carried out in a total assay volume of 0.5 ml with the same buffers as those used for isolation of membranes. Concentrations of [^{3}H]-glibenclamide of 0.01 nM to 20 nM were used for K_D and B_{max} determinations whilst 0.5 nM was used for drug competition studies in rat brain and HIT-T15 β-cell homogenates and 2 nM for guinea-pig ventricular homogenates. Drugs were dissolved in ethanol with the exception of BTS 67 582 which was dissolved in assay buffer. Nonspecific binding in the assay was assessed by use of unlabelled glibenclamide (30 μM). The assay tubes were allowed to equilibrate for 90 min at room temperature. The assay was stopped by rapid filtration through Whatman GF/B filters and washing with 5 ml of ice-cold assay buffer with a Skatron cell harvester. Dried filters were submerged in 5 ml scintillant (Packard 299) and counted in a liquid scintillation counter (Packard TriCarb 2100 TR).

Drugs

BTS 67 582, as the fumarate salt, was synthesized by the Medicinal Chemistry Department, Knoll Pharmaceuticals, Nottingham, and doses presented here are based on the weight of this salt. Diazoxide, glibenclamide, chlorpropamide and tolbutamide were purchased from Sigma, and [^{3}H]-glibenclamide (51 mCi $mmol^{-1}$) was purchased from NEN. Cromakalim was kindly supplied by SmithKline Beecham (U.K.). All other chemicals were obtained from Sigma or BDH and were of 'Analar' grade or higher.

Statistics and data analysis

All data are presented as the mean ± s.e.mean. Dose-response studies in animals were analysed by two way analysis of cov-

ariance with baseline as a covariate and treatment and experiment as factors. The effects of BTS 67 582 were compared to controls by use of William's test. IC_{50} values for binding experiments and rat aortic strip experiments were determined by the use of non-linear regression analysis (P-fit computer package, Elsevier). pA_2 values were calculated from a Schild plot (Arunlakshana & Schild, 1959). The K_D and B_{max} values were estimated by use of the Enzfitter computer package (Elsevier) or the EBDA/LIGAND computer package if two binding sites were present.

Results

Studies in fasted rats

In vehicle-treated fasted rats, baseline plasma glucose (4.9 ± 0.13 mmol l^{-1}, $n=28$) and insulin (0.97 ± 0.08 ng ml^{-1}, $n=24$) were not significantly different from those of the drug treated groups. Plasma glucose fell slightly by a 3.8%, 8.3% and 4.7%, and plasma insulin varied slightly by 2.6%, −3.0% and 5.7%, at 1, 2 and 4 h respectively, after administration of vehicle only.

BTS 67 582 (1 to 300 mg kg^{-1}) caused a time- and dose-dependent fall in plasma glucose (Figure 2a). The glucose lowering effect of BTS 67 582 was observed within 1 hr and was maximal between 2 to 4 h after oral administration. Significant differences from control ($P<0.05$, William's test) were observed with doses from 3 mg kg^{-1} and more than 1 h after dosing. ED_{50} values for BTS 67 582 1, 2 and 4 h after oral administration were 37.6 ± 12.6, 18.4 ± 4.1 and 18.5 ± 5.3 mg kg^{-1}, respectively. Although BTS 67 582 is the fumarate salt, sodium fumarate itself (100 mg kg^{-1} did not significantly affect plasma glucose (maximum fall of $4.1 \pm 3.0\%$) or insulin concentrations (maximum increase of $14.9 \pm 38.8\%$).

Tolbutamide (1 to 300 mg kg^{-1}, Figure 2b) and glibenclamide (0.003 to 100 mg kg^{-1}, Figure 2c) also caused time- and dose-dependent falls in plasma glucose with ED_{50} values at 1, 2 and 4 h after dosing of 11.5 ± 13.0, 13.7 ± 1.7 and 27.4 ± 2.1 mg kg^{-1} and 8.2 ± 15.1, 0.4 ± 0.1 and 0.4 ± 0.1 mg kg^{-1}, respectively (plasma glucose values were significantly different from control, $P<0.05$, from 1 mg kg^{-1} with tolbutamide and from 0.03 mg kg^{-1} with glibenclamide). Tolbutamide at 100 and 300 mg kg^{-1} caused a maximum fall in plasma glucose between 2 and 4 h after administration whereas at lower doses its glucose-lowering effect appeared to peak at 1 h and to diminish with time. Glibenclamide caused a progressive fall in plasma glucose during the experimental period. A comparison of dose-response relationships between BTS 67 582, tolbutamide and glibenclamide 2 h after administration is shown in Figure 3. BTS 67 582 and tolbutamide showed similar relatively steep dose-response curves, whereas glibenclamide exhibited a shallower dose-response. All three compounds caused a similar maximal percentage decrease in plasma glucose of about 50%.

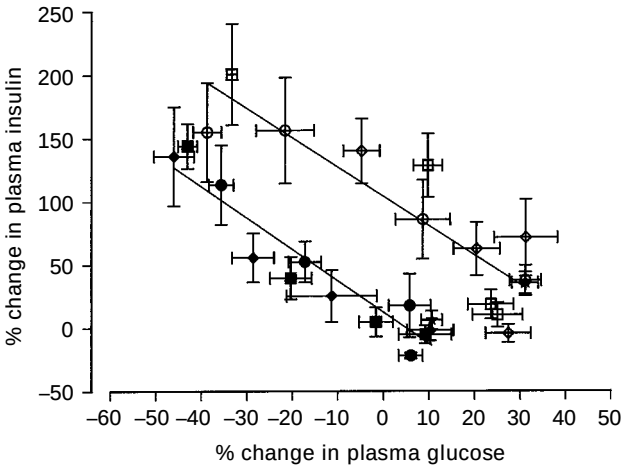

Figure 6 Relationship between percentage change in plasma glucose and plasma insulin in normal fasted glucose loaded rats administered either vehicle (★), BTS 67 582 10, 30 and 100 mg kg^{-1} (■,□), tolbutamide 10, 30 and 100 mg kg^{-1} (●, ○) or glibenclamide 0.1, 1 and 10 mg kg^{-1} (◆, ◇), 1 h (open symbols) and 2 h (closed symbols) after dosing.

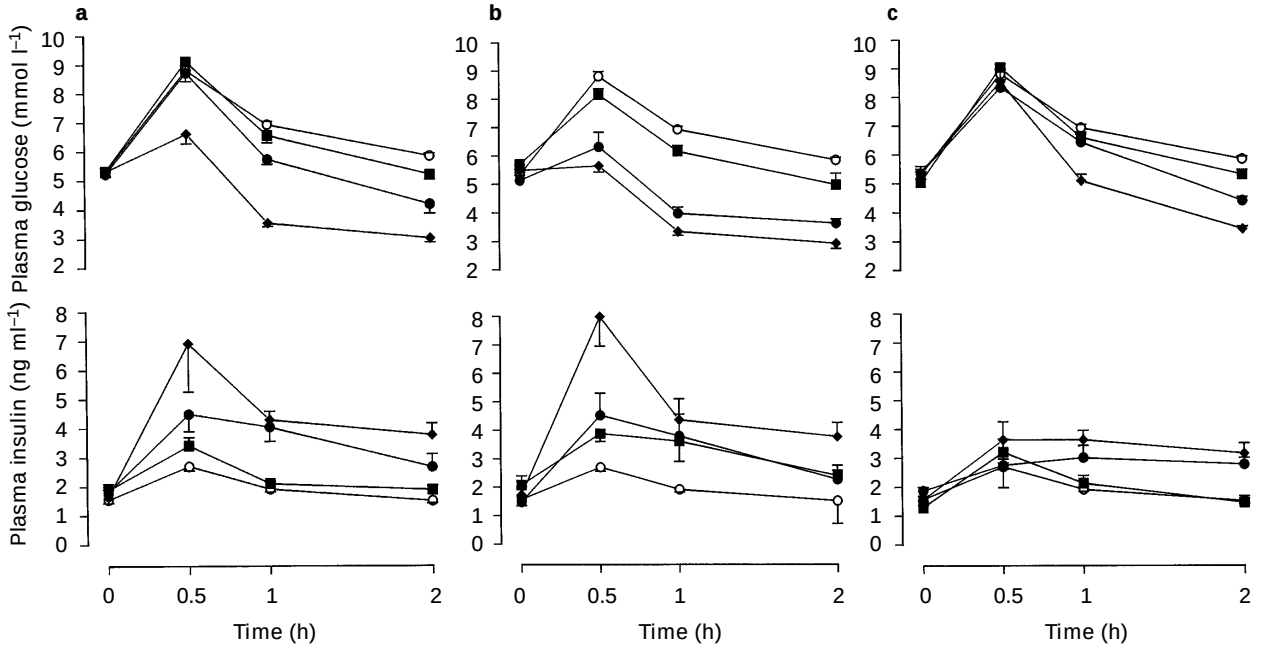

Figure 5 Effect of (a) BTS 67 582 at 100 (◆), 30 (●) and 10 (■) mg kg^{-1} (b) tolbutamide 100 (◆), 30 (●) and 10 (■) mg kg^{-1}, and (c) glibenclamide 10 (◆), 1.0 (●) and 0.1 (■) mg kg^{-1}, on plasma glucose and insulin in glucose loaded normal fasted rats. $n=6-8$, except vehicle (○) where $n=31$. Both the glucose load (800 mg kg^{-1}, s.c.) and drugs (p.o.) were dosed at 0 hour. Vertical lines show s.e.mean.

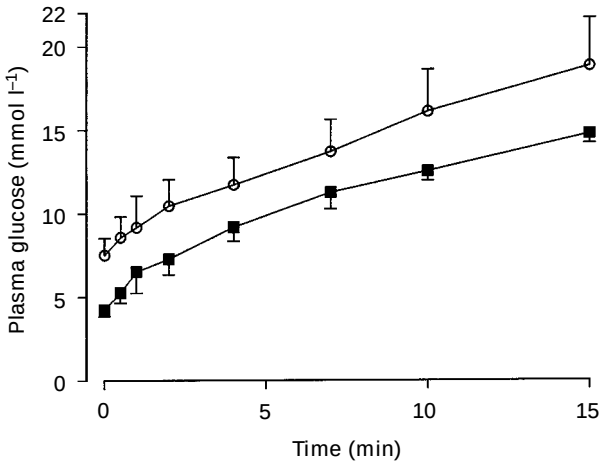

Figure 7 Effect of BTS 67 582 at 100 (■) mg kg^{-1} p.o. dosed at −2 h and vehicle (○) on the initial rate of glucose absorption into the hepatic portal vein following a glucose load (2 g kg^{-1} i.j.) in anaesthetized normal fasted rats ($n=5$). Vertical lines show s.e.mean.

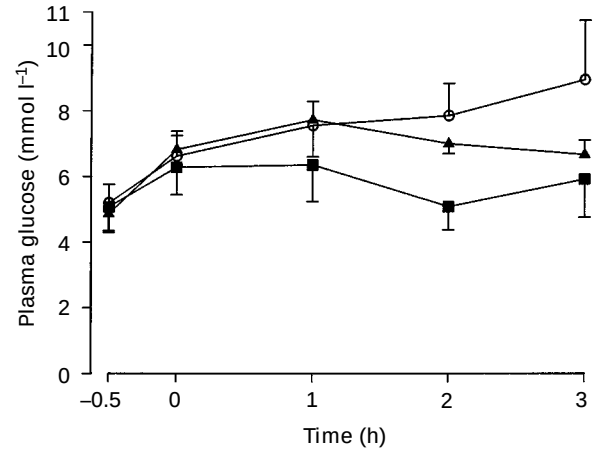

Figure 8 Effect of BTS 67 582 100 mg kg^{-1} (■), glibenclamide 1 mg kg^{-1} (▲) on diazoxide (30 mg kg^{-1})-induced (○) hyperglycaemia in normal fasted rats ($n=5$). Vertical lines show s.e.mean.

BTS 67 582 increased plasma insulin (significantly different from control, $P<0.05$, at doses of 10 mg kg^{-1} and above); this effect peaked between 1 and 2 h after administration and declined thereafter (Figure 2a). Tolbutamide caused a distinct peak in plasma insulin at 1 h with all doses which then fell rapidly (Figure 2b, significantly different from control, $P<0.05$, at doses of 30 mg kg^{-1} and above). Glibenclamide caused peak plasma insulin levels between 1 and 2 h after administration which were sustained during the experimental period (Figure 2c, significantly different from control, $P<0.05$, at doses of 1 mg kg^{-1} and above).

In a supplementary experiment in normal fasted Wistar rats, a longer term temporal response (up to 8 h after dosing) was examined to investigate the apparent changes in pharmacodynamics of glibenclamide and tolbutamide with dosage and to compare them with BTS 67 582 (Figure 4). The doses used were (a) an approximate ED_{50} and (b) the maximal glucose-lowering dose derived from the previous dose-response study. The glucose lowering effect of BTS 67 582 (100 and 30 mg kg^{-1}) was maximal between 2 to 4 h and both doses returned to baseline values at the same time, 6 h after dosing. Tolbutamide at both 30 and 100 mg kg^{-1} showed a maximal effect at 2 h returning to baseline at 4 h and 6 h, respectively. Glibenclamide 10 mg kg^{-1} produced a progressive fall in plasma glucose throughout the experiment whereas at 0.5 mg kg^{-1} recovery towards baseline was evident after 2 h.

BTS 67 582 (37 mg kg^{-1}) when administered i.p. caused a significantly greater percentage fall in plasma glucose initially but otherwise its glucose-lowering effect was similar to the oral route. This indicates that BTS 67 582 is well absorbed (falls in plasma glucose 1, 2 and 4 h after oral and i.p. administration were $14.5\pm4.6\%$, $36.6\pm4.2\%$ and $33.9\pm4.1\%$ ($n=8$) and $49.3\pm3.3\%$*, $34.1\pm3.6\%$ and $23.0\pm4.5\%$ ($n=8$), respectively); asterisk denotes significantly ($P<0.05$) different from corresponding value after oral administration (Student's t test). In addition, no evidence of tachyphylaxis of the glucose-lowering effects of BTS 67 582 (37 mg kg^{-1}) was observed (fall in plasma glucose two hours after administration of $37.4\pm3.0\%$ and $33.3\pm3.2\%$ on day 5 and day 1, respectively).

Studies in glucose-primed rats

In vehicle-treated rats ($n=31$), a glucose load caused plasma glucose to rise from 5.32 ± 0.08 to 8.81 ± 0.17 mmol l^{-1} 0.5 h after administration which then fell to 6.93 ± 0.11 and 5.85 ± 0.09 mmol l^{-1}, respectively, 1 and 2 h after administration. BTS 67 582 (Figure 5a) at 3 and 10 mg kg^{-1} did not affect plasma glucose, although at 30 mg kg^{-1} a reduction in glucose was seen at 1 and 2 h but not at 0.5 h after administration. BTS 67 582 at 100 mg kg^{-1} caused a marked reduction in plasma glucose at all time points. BTS 67 582 did not cause any significant changes in plasma insulin at 10 mg kg^{-1}, but at 30 and 100 mg kg^{-1} plasma insulin was dose-dependently increased.

Tolbutamide caused dose-dependent falls in plasma glucose at 10, 30 and 100 mg kg^{-1}, the rise in plasma glucose being virtually abolished at the highest dose used (Figure 5b). Tolbutamide caused a prompt increase in plasma insulin 0.5 h after 10, 30 and 100 mg kg^{-1}. None of the glibenclamide doses caused a fall in plasma glucose 0.5 h after administration and only the top dose (10 mg kg^{-1}) caused a reduction at 1 h after administration (Figure 5c). However, by 2 h after administration a dose-dependent reduction in plasma glucose was evident. No significant changes in plasma insulin were observed 0.5 h after administration with glibenclamide although some elevations were observed at 1 and 2 h.

Data from the BTS 67 582, tolbutamide and glibenclamide dose-response studies were combined and a highly significant correlation was observed between the percentage change in plasma insulin and plasma glucose 1 and 2 h after administration ($r=0.89$, $P<0.001$, and $r=0.94$ $P<0.0001$, respectively, Figure 6). A significant correlation was also observed 0.5 h after administration, although this was somewhat weaker ($r=0.79$, $P<0.05$). This is consistent with a causal relationship between elevated plasma insulin and the reduction of plasma glucose. The relationship between stimulation of insulin and the reduction of plasma glucose was similar for the three agents at these times.

Intestinal absorption of glucose

Baseline plasma glucose in the hepatic portal vein was lower 2 h after BTS 67 582 (100 mg kg^{-1}, i.j.) than that observed in vehicle-treated rats (4.26 ± 0.41 mmol l^{-1} and 7.52 ± 1.00 mmol l^{-1}, $n=5$, respectively), the percentage fall (43%) being consistent with studies presented above. After glucose loading, the rise in plasma glucose in the hepatic portal vein was similar in both vehicle and BTS 67 582 treated rats and indicates that the initial rate of absorption was not influenced by BTS 67 582 (Figure 7). This study was not extended beyond 15 min after glucose loading due to the influence of BTS 67 582 on glucose disposal.

Diazoxide-induced hyperglycaemia

In normal Wistar rats, diazoxide (30 mg kg^{-1}, i.p.) induced hyperglycaemia. Plasma glucose rose progressively from a baseline value of 5.2 ± 0.56 to 8.95 ± 1.8 mmol l^{-1} ($n=8$), 3 h

after administration (Figure 8). BTS 67 582 (100 mg kg^{-1}) antagonized the hyperglycaemia induced by diazoxide, plasma glucose values being significantly different 2 h after administration ($P<0.05$, Student's t test). Glibenclamide (1 mg kg^{-1}) also antagonized the effect of diazoxide although the effect was weaker and slower to develop. Plasma insulin in this experiment was reduced to below the level of detection of the insulin assay.

Cromakalim-induced relaxation

Cromakalim caused relaxation of noradrenaline (10^{-8} M) contracted rat aortic strips with an IC_{50} of approximately 5×10^{-8} M. Glibenclamide antagonized the effects of cromakalim-induced relaxation of noradrenaline contracted rat aortic strips, a pA_2 value of 7.47 (Hill slope = 1.08) was calculated (Figure 9a). BTS 67 582 also antagonized the effects of cromakalim although it was substantially weaker ($pA_2 = 5.88$, Hill slope = 0.81, Figure 9b).

Studies in streptozotocin-induced diabetes in rats

Streptozotocin (STZ) caused plasma glucose in fasted rats to increase from approximately 5 to 22.6 mmol l^{-1} indicating that the animals were moderately diabetic. In vehicle-treated STZ rats, an oral glucose load raised plasma glucose from 22.3 ± 0.5 to 35.2 ± 0.8, 29.6 ± 0.9 and 24.8 ± 1.1 mmol l^{-1} ($n=44$) at 0.5, 1.5 and 3.5 h after administration, respectively, Glibenclamide (100 mg kg^{-1}) caused a slight but not significant fall in plasma glucose in STZ diabetic rats. Plasma glucose values were 22.2 ± 0.5, 32.5 ± 0.7, 27.7 ± 0.8 and 22.6 ± 1.1 mmol l^{-1} ($n=44$), at 0.5, 1.5 and 3.5 h after administration, respectively. This corresponded to a 7.7%, 6.4% and 8.9% fall in plasma glucose which did not reach conventional levels of significance ($P=0.09$, 0.10 and 0.17, respectively, Student's t test) compared to corresponding control values.

The baseline plasma glucose values for animals administered BTS 67 582 at 37, 73 and 147 mg kg^{-1} were 23.0 ± 0.6

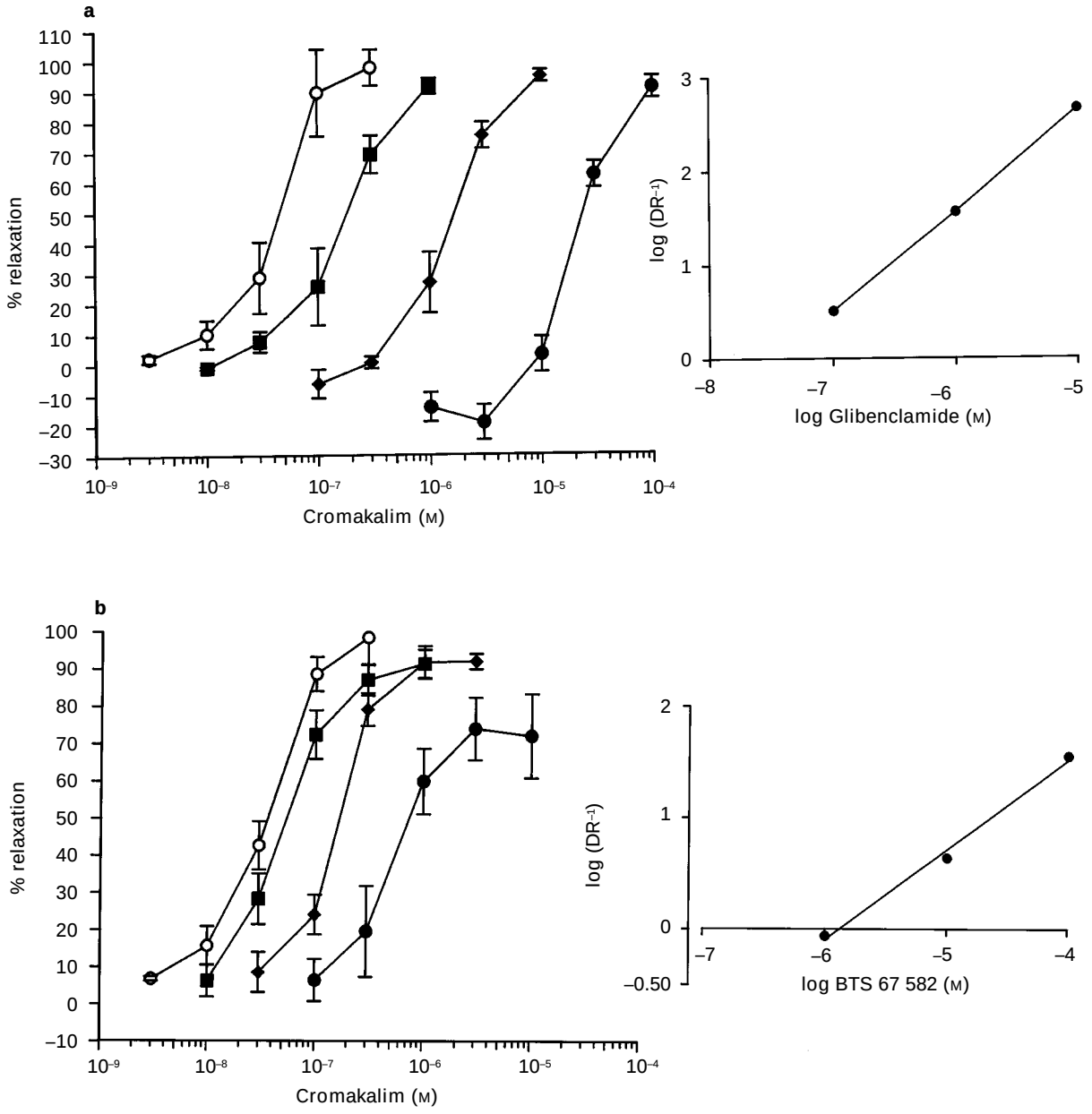

Figure 9 Effect of BTS 67 582 (■ 10^{-6}, ◆ 10^{-5} and ● 10^{-4} M, b) and glibenclamide (■ 10^{-7}, ◆ 10^{-6}, ● 10^{-5} M, a) on cromakalim-induced relaxation of noradrenaline (○, 10^{-8} M) contracted rat aortic strips ($n=4-6$). Vertical lines show s.e.mean. Insets: Schild plots.

($n=16$), 23.8 ± 0.4 ($n=31$) and 22.2 ± 0.4 mmol l^{-1} ($n=46$) and were not significantly different from control values 22.6 ± 0.3 mmol l^{-1} ($n=80$). BTS 67 582 at all doses used caused significant reductions ($P<0.01$, William's test) in plasma glucose compared to control values (Figure 10). The average reduction in plasma glucose (adjusted for differences between treatment groups at baseline and differences between experiments) was 12.6%, 18.3% and 16.1% at 37, 73 and 147 mg kg^{-1} BTS 67 582, respectively.

Plasma insulin was not routinely measured in glucose loaded STZ rats but in a separate experiment, plasma insulin was shown to be significantly raised by BTS 67 582 (values being 0.42 ± 0.09, 0.90 ± 0.15* and 0.65 ± 0.08 ng ml^{-1} 1 h after administration ($n=7-8$) for vehicle, BTS 67 582 (293 mg kg^{-1}) and glibenclamide (100 mg kg^{-1}), respectively, *$P<0.05$ versus vehicle).

In a separate series of experiments BTS 67 582 had no effect on the urinary excretion of glucose in STZ rats. Vehicle-treated STZ rats had an initial plasma glucose of 21.5 ± 0.85 mmol l^{-1} which rose to 33.3 ± 1.01 and 19.3 ± 1.41 mmol l^{-1} ($n=8$), 1 and 3.5 h after glucose loading. In STZ rats administered BTS 67 582 (293 mg kg^{-1}), initial plasma glucose was similar to controls (21.7 ± 0.82), but was lower at 1 h (25.8 ± 2.36 mM, $P<0.05$ compared to vehicle, Student's t test) and 3.5 h (15.6 ± 1.58, $n=8$) following the glucose load. Urine volume (1.79 ± 0.36 and 2.06 ± 0.21 ml urine/rat) and glucose content (0.110 ± 0.025 and 0.122 ± 0.037 g glucose/rat) in BTS 67 582 and vehicle-treated STZ rats, respectively, were not significantly different from each other in urine collected from 1 to 3.5 h after dosing. Similarly no difference was noted in the volume or glucose content of urine collected between 3.5 and 6.5 h after dosing, urine volume being 0.65 ± 0.06 and 0.63 ± 0.09 ml/rat and urine glucose content being 0.010 ± 0.004 and 0.009 ± 0.003 g glucose/rat for vehicle and BTS 67 582 treated rats, respectively.

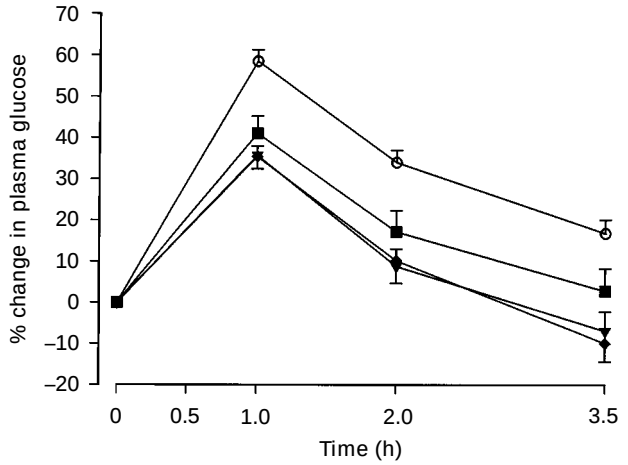

Figure 10 Effect of BTS 67 582 (■, 37 mg kg^{-1}, $n=16$; ▼, 73 mg kg^{-1}, $n=31$; ◆, 147 mg kg^{-1}, $n=46$) and vehicle (○, $n=80$) on percentage reduction in plasma glucose in fasted streptozotocin diabetic rats given a glucose load (800 mg kg^{-1}, p.o.). Vertical lines show s.e.mean.

[^{3}H]-glibenclamide binding studies

Binding of [^{3}H]-glibenclamide to rat brain and HIT-T15 β-cell homogenates was complex and EBDA/LIGAND analysis indicated the presence of both high and low affinity binding sites (Table 1). In contrast, in a variety of membrane preparations, the binding of [^{3}H]-glibenclamide to guinea-pig ventricle homogenates showed binding consistent with only a single low affinity binding site (Table 1). Glibenclamide, tolbutamide and chlorpropamide displaced [^{3}H]-glibenclamide from its binding site in all three membrane preparations, whilst BTS 67 582 caused less than 10% displacement of binding when tested at concentrations of up to 1 mM (Table 2). The presence of MgATP or MgADP at 5 mM in the assay with rat brain membranes did not influence the effect of BTS 67 582.

Discussion

BTS 67 582 caused time- and dose-dependent falls in plasma glucose with corresponding increase in plasma insulin in normal rats. The onset of this effect with BTS 67 582 appears to be intermediate between the rapid onset of action of tolbutamide and the comparatively slow onset of action of glibenclamide, an observation most noticeable during glucose loading. In an extended temporal response study it was noted that at high doses, both tolbutamide and glibenclamide appear to have a greater duration of action than that observed at lower doses. This effect has been shown previously for glibenclamide, although not for tolbutamide (Holmes *et al.*, 1984). In addition, a shallower dose-response curve of glibenclamide compared

Table 1 Kinetics of [^{3}H]-glibenclamide binding to crude membrane fractions of rat brain, guinea-pig ventricle and HIT-T15 β-cell line

	High affinity site		*Low affinity site*	
Tissue	K_d (nM)	B_{max} (pmol mg^{-1})	K_d (nM)	B_{max} (pmol mg^{-1})
Rat brain	0.14 ± 0.02	0.065 ± 0.003	340 ± 140	2.6 ± 0.9
Guinea-pig ventricle	–	–	110	5.0
HIT-T15 β-cells	0.15 ± 0.10	0.062 ± 0.024	11.0 ± 6.0	5.1 ± 4.5

Binding kinetic studies were conducted in triplicate with three separate membrane preparations except for guinea-pig ventricle where a single representative experiment is shown.

Table 2 IC_{50} values for the displacement of [^{3}H]-glibenclamide by antidiabetic drugs

	HIT-T15 β-cell line	*Guinea-pig ventricle*	*Rat brain*
BTS 67 582	<10% (1 mM)	<10% (1 mM)	<10% (1 mM)
Glibenclamide	2.1 ± 0.3 nM	230 ± 30 nM	1.4 ± 0.1 nM
Tolbutamide	37 ± 4 μM	11 ± 2 μM	37 ± 2 μM
Chlorpropamide	110 ± 20 μM	130 ± 40 μM	160 ± 10 μM

Studies for the rat brain and HIT-T15 membranes were conducted with an isotope concentration of 0.5 nM and data were derived from three separate membrane preparations. Studies in guinea-pig ventricle membranes were conducted with an isotope concentration of 2 nM and data derived from 1–3 separate membrane preparations.

with that of either tolbutamide or BTS 67 582 was also observed in this study. Second generation sulphonylureas, such as glibenclamide, tend to accumulate in the pancreatic β-cell and are known to have a relatively slow kinetic action due to their lipophilic nature and the competition between receptor binding and intracellular accumulation (Panten *et al*., 1989; Schwanstecher *et al*., 1994). In comparison, BTS 67 582 appears to have a similar duration of action when administered at either maximal or approximate ED_{50} doses, which may be due to its hydrophilic and basic physiochemical properties.

The strong correlation between percentage change in plasma glucose and insulin observed in glucose-primed normal rats, indicates that stimulation of insulin secretion is the prime mechanism of action of BTS 67 582, tolbutamide and glibenclamide in this study. This was further supported by experiments in normal rats which indicated that BTS 67 582 did not interfere with glucose uptake from the gastrointestinal tract and experiments in streptozotocin-induced diabetic rats where BTS 67 582 did not enhance urinary excretion of glucose. Thus insulin-stimulated glucose disposal appears to be the primary mechanism of the plasma glucose lowering action of BTS 67 582.

Streptozotocin is toxic to β-cells and has been widely used to induce diabetes in animals (Cooperstein & Watkins 1981; Bailey & Flatt 1990). A dose of streptozotocin was chosen in this study so that not all β-cells were destroyed. This was achieved since exogenous insulin administration was not required and measurable levels of circulating insulin were observed and is therefore considered a moderate to severe model of NIDDM. BTS 67 582 caused significant falls in plasma glucose in glucose loaded STZ rats whereas glibenclamide did not cause significant falls. This improvement in glycaemic control caused by BTS 67 582 was most likely due to a greater, and significant, stimulation of insulin secretion in STZ rats than that caused by glibenclamide, although the mechanism of this effect is at present unclear.

The binding parameters of [^{3}H]-glibenclamide in rat brain and HIT-T15 β-cell membrane preparations in this study are consistent with those obtained by Zini *et al*. (1991) for rat brain, and French *et al*. (1991) for insulinoma cells. However, in other studies only a single binding site in these tissues has been found (Gaines *et al*., 1988; Robertson *et al*., 1990). The variability in [^{3}H]-glibenclamide binding to various tissues has recently been reviewed (Ashcroft & Ashcroft, 1992; Edwards & Weston, 1993). The presence of only a single low affinity binding site in the guinea-pig ventricle preparation used in this study contrasts with data in the literature (Fosset *et al*., 1988; French *et al*., 1990; 1991). The reason for the identification of only a single low affinity binding site in this study in ventricle is not clear. Since BTS 67 582 showed no tendency to displace [^{3}H]-glibenclamide from its binding site in any of the membrane preparations tested, in this respect it can be clearly differentiated from the sulphonylureas.

Although BTS 67 582 does not appear to interact at the same binding site as glibenclamide, it may nevertheless inhibit potassium channel activity via other mechanisms. The effect of the potassium channel opener diazoxide on insulin secreting cells has been extensively characterized and has been shown to have essentially opposite effects to the anti-diabetic K_{ATP} channel blockers, such as tolbutamide. Thus diazoxide activates the K_{ATP} channel and inhibits insulin release through repolarization of the β-cell membrane (Henquin & Meisner, 1982; Trube *et al*., 1986; Dunne, 1989). The glucose lowering effect of BTS 67 582 and glibenclamide in this study was inhibited by diazoxide. This suggests that modulation of β-cell K_{ATP} channel activity by BTS 67 582 may be involved in its mechanism of action. The hyperglycaemic action of diazoxide appeared to be more rapidly and more effectively antagonized by BTS 67 582 than by glibenclamide. Glibenclamide has been observed previously to inhibit the hyperglycaemic activity of diazoxide in hypertensive rats (Clapham *et al*., 1994).

In other studies to address its mechanism of action, BTS 67 582 was found to antagonize the cromakalim-induced relaxation of noradrenaline-contracted rat aortic strips, providing further functional evidence that K_{ATP} channel blockade may play a role in the activity of this compound. The vasorelaxant effects of cromakalim and the antagonism of its actions by glibenclamide in rat aorta were consistent with results from other studies (Buckingham *et al*., 1989; Bray & Quast, 1992).

The sulphonylureas are in widespread use for the treatment of NIDDM and, indeed, are the only class of compounds currently used clinically as insulin secretagogues. Numerous studies have shown that the sulphonylureas act primarily by blocking the K_{ATP} channel in pancreatic islets leading to membrane depolarization, influx of calcium through voltage-dependent calcium channels and hence insulin secretion (Ashcroft & Ashcroft, 1992; Edwards & Weston, 1993). All sulphonylureas displace [^{3}H]-glibenclamide from its binding site and their hypoglycaemic activity is strongly correlated to their ability to cause displacement of [^{3}H]-glibenclamide from its binding sites (Geisen *et al*., 1985; Schmid-Antomarchi *et al*., 1987).

BTS 67 582 can be clearly differentiated from the sulphonylurea class of agents since it possesses a novel chemical structure, it does not displace [^{3}H]-glibenclamide from its binding site and it reduces plasma glucose in STZ rats which do not respond to glibenclamide. However, antagonism of cromakalim-induced relaxation of rat aortic strips and the antagonism of diazoxide-induced hyperglycaemia suggest that BTS 67 582 possesses functional potassium channel blockade. The exact mechanism whereby BTS 67 582 modulates potassium channel activity remains to be determined. In conclusion, BTS 67 582 is a novel and effective glucose-lowering agent and insulin releasing agent in both normal and streptozotocin-induced diabetic rats.

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