British Journal of Pharmacology (1997) 122, 1464–1468

Glucose-lowering effect of BTS 67 582

T. Page & [1]C.J. Bailey

Department of Pharmaceutical & Biological Sciences, Aston University, Birmingham B4 7ET

1 The hypoglycaemic effect of BTS 67 582 (1,1-dimethyl-2(2-morpholinophenyl) guanidine fumarate) was studied in normal rats.

2 BTS 67 582 (100 mg kg^{-1}, p.o.) acutely lowered basal plasma glucose concentrations: onset within 1 h, maximum decrease of >40% at 2–3 h, and partial return to euglycaemia by 5 h. Plasma insulin concentrations were increased: onset within 30 min, maximum increase 3 fold at 1–2 h; returning to normal by 5 h.

3 BTS 67 582 (100 mg kg^{-1}) increased (by 56%) the rate of disappearance of plasma glucose during an intravenous glucose tolerance test, accompanied by a 51% increase in insulin concentrations.

4 During hyperglycaemic clamp studies BTS 67 582 (100 mg kg^{-1}) increased glucose utilization 3 fold. This was associated with a 3 fold increase in insulin concentrations, even in the presence of adrenaline at a dosage which inhibits glucose-induced insulin release.

5 When the insulin-releasing effect of BTS 67 582 (100 mg kg^{-1}) was inhibited by infusion of somatostatin, there was no effect on glycaemia.

6 Insulin-dependent diabetic BB/S rats, which do not produce endogenous insulin, showed no effect of BTS 67 582 (100 mg kg^{-1}) on plasma glucose concentrations in the presence or absence of exogenous insulin.

7 The results demonstrate an acute hypoglycaemic effect of BTS 67 582 which appears to result mainly from its potent insulin-releasing action.

Keywords: BTS 67 582; dimethylmorpholinophenylguanidine; glucose tolerance; glucose utilization; insulin; lactate; diabetic BB/S rats

Introduction

BTS 67 582 (1,1-dimethyl-2(2-morpholinophenyl)guanidine fumarate) has recently been shown to act as a novel blood glucose-lowering agent which stimulates insulin secretion (Jones *et al*., 1997). In fasted and glucose-loaded normal rats, BTS 67 582 reduced glucose concentrations and increased insulin concentrations in a dose-dependent manner. BTS 67 582 also lowered glucose concentrations in streptozotocin (STZ)-induced non-insulin-dependent diabetic rats which did not respond to the sulphonylurea glibenclamide. Indeed, BTS 67 582 did not displace [^{3}H]-glibenclamide binding to cultured insulin-secreting HIT-T15 cells. Nevertheless, preliminary evidence indicates that BTS 67 582, like sulphonylureas, stimulates insulin secretion through closure of adenosine 5′-triphosphate (ATP)-sensitive potassium channels, causing depolarization and an acceleration of voltage-dependent calcium influx (Jones *et al*., 1996).

The present study was undertaken to investigate further the blood glucose-lowering effect of BTS 67 582. Glucose utilization has been quantified in normal rats during standard tests of glucose homeostasis including a hyperglycaemic hyperinsulinaemic clamp procedure. The possibility that BTS 67 582 might exert extra-pancreatic effects has been examined by suppression of insulin release with somatostatin, and by using insulin-dependent diabetic BioBreeding (BB/S) rats in which there is no remaining endogenous insulin secretion.

Methods

Animals

Adult male Wistar rats weighing 150–250 g were maintained as previously described (Bailey *et al*., 1992). Anaesthesia was induced with sodium pentobarbitone (60 mg kg^{-1}, i.p.) and maintained with further doses (15 mg kg^{-1}) as required. Rectal temperature was held at 34–36°C. Cannulae were introduced into the right jugular vein and right femoral vein for i.v. administration of test substances.

Diabetic BB/S (BioBreeding/Southampton) rats weighing approximately 300 g were obtained from the SPF colony bred at the Biomedical Research Facility, University of Southampton. The BB/S rats had no detectable endogenous insulin and were insulin-dependent for >50 days before study. The rats were maintained by subcutaneous daily administration of insulin (typically 2–6 units day^{-1}, Ultratard). Body weight, urinary glucose and ketone concentrations were measured semiquantitatively on alternate days (N-Labstix, Ames Division, Bayer Diagnostics, Slough). Insulin dosage was adjusted to achieve weight gain, avoid ketonuria and limit glycosuria to no more than trace positive. Plasma glucose was checked weekly. As noted previously (Bailey *et al*., 1987), withdrawal of insulin results in glycosuria, severe hyperglycaemia and ketonaemia. If untreated, this culminates in coma and death within days.

In the present studies BTS 67 582 was administered either orally or intrajejunally at a dosage of 100 mg kg^{-1}. This is a maximally effective dosage for the blood glucose-lowering effect at 2 h after oral administration to fasted normal rats (ED_{50} value 18.4±4.1 mg kg^{-1}, Jones *et al*., 1997).

Studies in normal rats

Basal glycaemia Twenty-four hours fasted conscious rats received BTS 67 582 (100 mg kg^{-1}, p.o.) dissolved in PBS, and blood samples (80 μl) were taken from the tail at intervals of up to 5 h. Plasma was separated for determination of glucose and insulin.

Hyperglycaemic hyperinsulinaemic infusion Rats were fasted for 24 h, anaesthetized and infused i.v. with glucose (16 mg kg^{-1} min^{-1}), insulin (2.7 mu kg^{-1} min^{-1}), adrenaline bitartrate (0.08 μg kg^{-1} min^{-1}) and propranolol hydrochloride (1.7 μg kg^{-1} min^{-1}) as described by Reaven *et al*., (1983). This produced a steady hyperglycaemia (about 12 mmol l^{-1})

[1] Author for correspondence.

and hyperinsulinaemia (about 4 ng ml^{-1}) by 90 min. This was maintained throughout the duration of the study (210 min) in the control animals. The effect of BTS 67 582 was determined by administration of the drug at a dosage of 100 mg kg^{-1} into the proximal jejunum at 90 min. Control animals received an equivalent volume of PBS, 10 ml kg^{-1}. Blood samples were taken from the tail at 30 min intervals for determination of plasma glucose and insulin concentrations. At 210 min simultaneous multiple site blood sampling was undertaken from the lower abdominal aorta, lower abdominal inferior vena cava (IVC) and the right iliac vein for determination of plasma glucose and lactate.

Hyperglycaemic hyperinsulinaemic clamp Rats were fasted for 24 h, anaesthetized and an i.v. infusion of insulin, adrenaline and propranolol was given as above, but the rate of glucose infusion was varied to maintain hyperglycaemia at 12 mmol l^{-1}. When steady rate conditions were achieved, rats received either BTS 67 582 (100 mg kg^{-1}) or vehicle only (PBS 10 ml kg^{-1}) delivered intrajejunally. Blood samples were taken from the tail at 15 min intervals for determination of plasma glucose and at 30 min intervals for plasma insulin. The steady state plasma glucose and insulin concentrations, and the amount of glucose infused to maintain the hyperglycaemia at 12 mmol l^{-1} were calculated 60–120 min after drug administration.

Somatostatin infusion Rats deprived of food for 24 h were anaesthetized and received an i.v. infusion of somatostatin (1 μg kg^{-1} min^{-1}), based on the procedure used in dogs (Cherrington *et al*., 1978). After 30 min, rats received either BTS 67 582 (100 mg kg^{-1}) or vehicle only (PBS 10 ml kg^{-1}) delivered intrajejunally. Blood samples were taken from the tail at 30 min intervals until 210 min for analysis of plasma glucose and insulin.

Intravenous glucose tolerance Rats were fasted for 24 h, anaesthetized and given either BTS 67 582 (100 mg kg^{-1}) or vehicle only (PBS 10 ml kg^{-1}) delivered intrajejunally. After 60 min rats received an i.v. injection of glucose (0.5 g kg^{-1} in a 20% w/v solution), and blood samples for plasma glucose determination were taken from the tail at 0, 60, 65 and 90 min. The rate of plasma glucose disappearance (% min^{-1}) was determined ($69.3/t_{1/2}$) between 65 and 90 min.

Studies in diabetic BB/S rats

Basal hyperglycaemia BB/S rats were deprived of exogenous insulin for 48 h and fasted overnight before experimentation. BTS 67 582 (100 mg kg^{-1}, p.o.) was administered to conscious rats and blood samples (80 μl) for plasma glucose and insulin determination were taken from the tail at intervals of up to 5 h.

Insulin hypoglycaemia test Overnight fasted BB/S rats which had been deprived of exogenous insulin for 48 h received BTS 67 582 (100 mg kg^{-1}, p.o.) 60 min before an i.p. injection of human insulin (Actrapid, 1 unit kg^{-1}). Blood samples for plasma glucose assay were taken from the tail at intervals of up to 120 min. In a separate study BTS 67 582 (100 mg kg^{-1}, p.o.) was given several hours after the insulin injection, when the plasma glucose concentration was rising. Blood samples for plasma glucose assay were taken from the tail at intervals up to 5 h.

Chemicals

All chemicals were obtained from Sigma Chemical Company, Poole or BDH, Poole, except: BTS 67 582 from Knoll Pharmaceuticals, Nottingham; Sagatal (sodium pentobarbitone) from RMB, Animal Health, Dagenham; PBS (phosphate buffered saline) tablets from Unipath (Basingstoke); Optiphase Hisafe II scintillant from Fisons (Loughborough); human Actrapid and Ultratard insulins, Novo Nordisk (Crawley); rat insulin standard from Novo Nordisk (Bagsvaerd, Denmark); anti-insulin serum from Linco Research (St. Louis, U.S.A.); and [A14-^{125}I]-insulin, from Amersham International (Amersham). Reagents for glucose analyses were from Beckman (High Wycombe) and reagents for lactate analyses were from Boehringer (Mannhiem, Lewes).

Analyses

Plasma glucose was measured by an automated glucose oxidase procedure (Stevens, 1971) and plasma lactate was measured by the lactate dehydrogenase method (Noll, 1974). Plasma insulin was determined by radioimmunoassay by means of a polyethylene glycol separation method and rat insulin as standard (Desbuquios & Aurbach, 1971).

Data are presented as mean ± s.e.mean. Differences between groups were compared by Student's unpaired t test. Differences were considered to be significant if $P<0.05$.

Results

Normal rats

Basal glycaemia Oral administration of BTS 67 582 (100 mg kg^{-1}) lowered plasma glucose concentrations in fasted conscious rats (Figure 1). The effect was evident by 1 h, was maximal at 2–3 h (42% decrease at 3 h versus time zero, $P<0.001$), and there was a partial return to euglycaemia by 5 h. Plasma insulin concentrations were raised by BTS 67 582 between 30 min and 3 h, with the maximum increase (>3 fold increase, $P<0.001$) at 1–2 h.

Hyperglycaemic hyperinsulinaemic infusion The infusion of glucose, insulin, adrenaline and propranolol in fasted anaesthetized rats created a steady hyperglycaemia (about

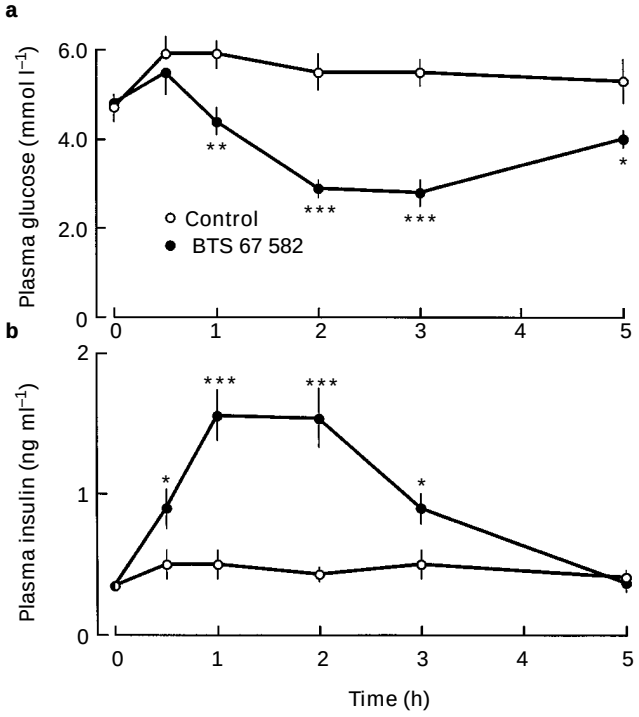

Figure 1 Effect of BTS 67 582 (100 mg kg^{-1}, p.o.) on basal plasma glucose (a) and insulin (b) concentrations in fasted conscious rats. Values are mean with s.e.mean shown by vertical lines; $n=6$. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ versus control, Student's unpaired t test.

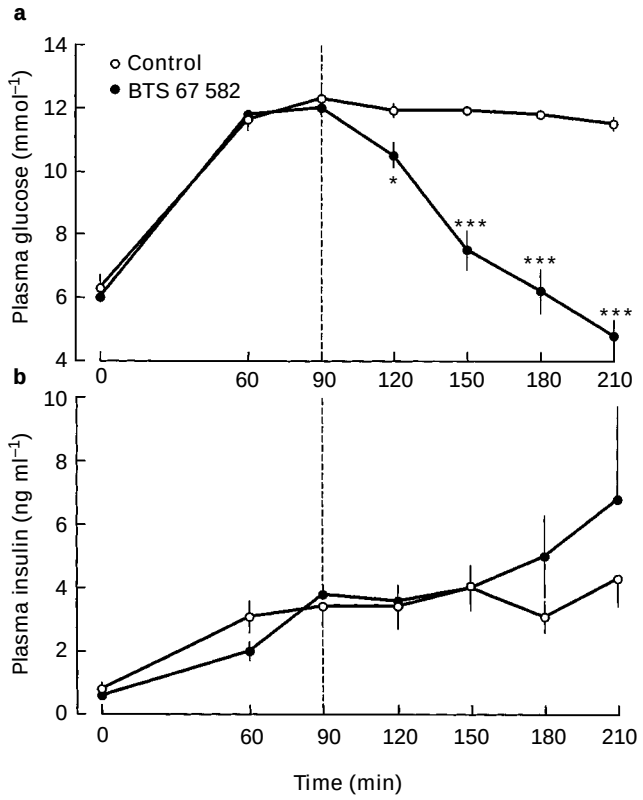

Figure 2 Plasma glucose (a) and insulin (b) concentrations of fasted anaesthetized rats during a hyperglycaemic hyperinsulinaemic infusion. At time 90 min, when a steady hyperglycaemia had been achieved, either 100 mg kg^{-1} BTS 67 582 or saline as control was given intrajejunally. Values are mean with vertical lines showing s.e.mean, $n=6$. $^{*}P<0.05$, $^{***}P<0.001$ versus control, Student's unpaired t test.

Table 1 Plasma glucose and lactate concentrations in the lower abdominal aorta, lower abdominal inferior vena cava (IVC) and right iliac vein at 210 min after a hyperglycaemic hyperinsulinaemic infusion in fasted anaesthetized rats

	Plasma glucose (mmol l^{-1})		*Plasma lactate* (mmol l^{-1})	
	Control	*BTS 67 582*	*Control*	*BTS 67 582*
	Concentration			
Aorta	12.9±0.3	8.0±0.6**	2.7±0.1	2.6±0.4
IVC	11.6±0.1	5.1±0.6**	2.1±0.1	2.4±0.2
Iliac vein	11.3±0.2	4.8±0.6**	2.0±0.2	1.9±0.1
	Arterio-venous difference			
Aorta-IVC	1.3±0.3	2.9±0.3*	0.5±0.1	0.2±0.3
Aorta-lliac vein	1.6±0.1	3.2±0.3*	0.7±0.1	0.7±0.3

Either 100 mg kg^{-1} BTS 67 582 or saline as control was given intrajejunally at 90 min. Means with s.e.mean are shown, $n=6$. $^{*}P<0.05$, $^{**}P<0.001$ versus control.

Table 2 Steady state plasma glucose and insulin concentrations, and glucose infusion rate during a hyperglycaemic hyperinsulinaemic clamp in fasted anaesthetized rats 60–120 min after intrajejunal administration of BTS 67 582 (100 mg kg^{-1})

	Control	*BTS 67 582*
SSPG (mmol l^{-1})	11.9±0.1	11.8±0.9
SSPI (ng ml^{-1})	4.6±0.7	17.6±1.4*
Glucose infused (μmol min^{-1} kg^{-1})	60.6±2.5	193.0±5.3*

SSPG, steady state plasma glucose; SSPI, steady state plasma insulin. Means with s.e.mean are shown, $n=6$. $^{*}P<0.001$ versus control.

12 mmol l^{-1}) and hyperinsulinaemia (about 4 ng ml^{-1}) between 90 and 210 min (Figure 2). Intrajejunal administration of BTS 67 582 (100 mg kg^{-1}) at 90 min resulted in a progressive decrease in the plasma glucose concentration. By 210 min (2 h after administration of BTS 67 582) the plasma glucose concentration was 58% lower than controls ($P<0.001$). This was associated with a rise in the mean circulating insulin concentration, due to a substantial increase in plasma insulin in some (but not all) animals. This raises the possibility that BTS 67 582 might have been increasing endogenous insulin release despite the administration of adrenaline and the lower plasma glucose concentrations.

Multiple site sampling of blood at 210 min showed that BTS 67 582 had approximately doubled the arterio-venous plasma glucose difference, which was measured across the lower limbs and iliac region (Table 1). Plasma lactate concentrations were also measured because BTS 67 582 is a guanidine derivative, and some guanidine-related compounds are known to stimulate conversion of glucose to lactate (Bailey, 1992). Arterial and venous plasma lactate concentrations at 210 min were not significantly affected by BTS 67 582 (Table 1).

Hyperglycaemic clamp A hyperglycaemic clamp in fasted anaesthetized rats was maintained at a steady plasma glucose concentration of about 12 mmol l^{-1}. The amount of glucose infused into rats treated intrajejunally with BTS 67 582 (100 mg kg^{-1}) was 3 fold greater than control rats (Table 2), demonstrating a marked increase in glucose utilization. Moreover, the plasma insulin concentration was also raised 3 fold in the group receiving BTS 67 582, indicating a substantial release of endogenous insulin in the hyperglycaemic state.

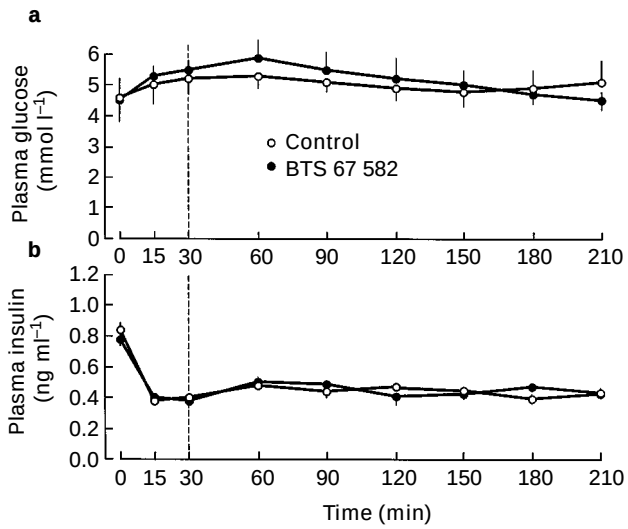

Figure 3 Plasma glucose and insulin concentrations of fasted anaesthetized rats during an intravenous infusion of somatostatin (1 μg kg^{-1} min^{-1}). At time 30 min either 100 mg kg^{-1} BTS 67 582 or saline as control was given intrajejunally. Values are mean with vertical lines showing s.e.mean, $n=6$.

Somatostatin infusion Infusion of somatostatin promptly reduced insulin concentrations in fasted anaesthetized rats, accompanied by a slight rise in glycaemia (Figure 3). Intrajejunal administration of BTS 67 582 (100 mg kg^{-1}) at 30 min did not significantly alter plasma glucose or insulin concentrations compared with control rats.

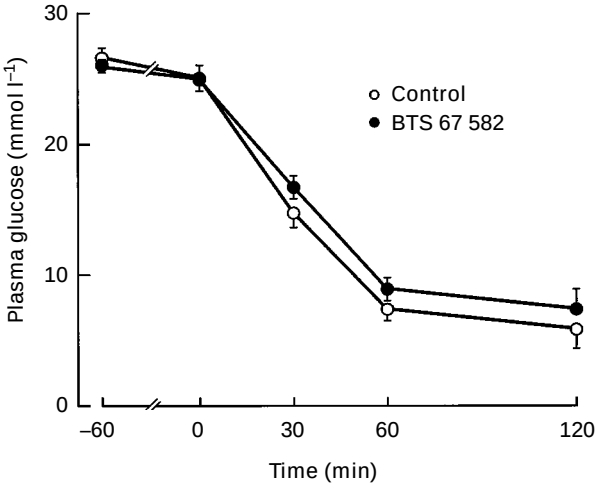

Figure 4 Effect of BTS 67 582 (100 mg kg^{-1}, p.o., at −60 min) on the plasma glucose response to insulin (1 unit kg^{-1}, i.p., at 0 min) in fasted conscious diabetic BB/S rats previously deprived of exogenous insulin for 48 h. Values are mean with vertical lines showing s.e.mean, $n=7$.

Intravenous glucose tolerance BTS 67 582 (100 mg kg^{-1}, intrajejunally) improved intravenous glucose tolerance. The rate of plasma glucose disappearance was 3.0 ± 0.2% min^{-1} in the group treated with BTS 67 582, compared with 1.9 ± 0.3 % min^{-1} in controls (56% increase; $P<0.05$). This was accompanied by raised plasma insulin concentrations after administration of BTS 67 582 (sum of insulin concentrations at 65 and 90 min was increased by 51%; $P<0.05$).

Diabetic BB/S rats

Basal hyperglycaemia BB/S rats deprived of exogenous insulin for 48 h showed a marked but stable hyperglycaemia (about 25 mmol l^{-1}). Plasma insulin was not detectable (<0.1 ng ml^{-1}) before or during the experiment. BTS 67 582 (100 mg kg^{-1}, p.o.) did not alter plasma glucose concentrations over 5 h (data not shown).

Insulin hypoglycaemia test BTS 67 582 (100 mg kg^{-1}, p.o.) did not alter the hypoglycaemic response to an i.p. injection of insulin in BB/S rats (Figure 4). In a separate study, administration of the BTS 67 582 several hours after the insulin injection did not alter the rise in plasma glucose concentrations (data not shown).

Discussion

This study has shown that the acute blood glucose-lowering effect of BTS 67 582 is strongly associated with its insulin-releasing effect. Increased insulin concentrations and increased glucose utilization were especially marked during a hyperglycaemic clamp. Conversely, glucose concentrations were unaffected by BTS 67 582 when insulin secretion was inhibited by somatostatin, and BTS 67 582 was ineffective against the hyperglycaemia of diabetic BB/S rats which lack endogenous insulin secretion.

There are various potential mechanisms, both direct and indirect, through which BTS 67 582 could enhance insulin release from B-cells of the pancreatic islets of Langerhans (Flatt, 1992). Such mechanisms could be dependent or independent of the insulin-releasing effect of glucose. Since BTS 67 582 raised insulin concentrations to a greater extent when hyperglycaemia was clamped (Table 2) than when glucose concentrations were allowed to fall (Figure 2), or when glucose concentrations were basal (Figure 1), it appears that BTS 67 582 exerts a greater insulin-releasing effect when blood glucose concentrations are raised. Preliminary studies measuring insulin secretion by perifused rat islets would support this view (Dickinson *et al*., 1997). Interestingly, adrenaline (0.08 μg kg^{-1} min^{-1}) did not eliminate an insulin-releasing effect of BTS 67 582 (100 mg kg^{-1}), confirming the potency of the insulinotropic effect at a dosage of adrenaline which inhibits glucose-induced insulin secretion (Reaven *et al*., 1983).

The blood glucose lowering effect of BTS 67 582 appears to result mainly from the stimulation of insulin release. Thus, inhibition of insulin secretion by somatostatin prevented the glucose-lowering effect of BTS 67 582. Moreover, insulin-dependent diabetic BB/S rats, with and without exogenous insulin treatment, failed to demonstrate any measurable extrapancreatic effect of BTS 67 582. While this does not absolutely exclude the possibility that some extrapancreatic activity might contribute to increased glucose utilization by BTS 67 582, such an effect certainly could not play a substantive role in the enhancement of glucose utlization.

Although BTS 67 582 is a guanidine derivative, its mode of action is distinctly different from that of biguanides, which are guanidine derivatives presently used in the treatment of non-insulin dependent diabetes mellitus (Bailey 1992; Bailey & Turner, 1996). Thus, unlike biguanides, BTS 67 582 does not have significant extrapancreatic activity and does not exert a hyperlactataemic effect, but does potently stimulate insulin release. Whether BTS 67 582 could influence glycosylation, as noted with aminoguanidine (Brownlee, 1994) has not been demonstrated.

Administration of BTS 67 582 to fasted rats lowers plasma glucose and raises plasma insulin with a similar potency to the sulphonylurea tolbutamide (Jones *et al*., 1997). Phase 1 clinical studies with BTS 67 582 have shown that it exerts a rapid-onset and short duration of action which can be timed to coincide with a single meal (Byrom *et al*., 1994; 1996). BTS 67 582 is structurally different from sulphonylureas, which are the main insulin-releasing agents used in the treatment of non-insulin dependent diabetes mellitus (Groop, 1992). Although both types of compound promote insulin release by closure of ATP-sensitive K^+-channels in the pancreatic B-cell membrane, evidence that BTS 67 582 does not displace the binding of glibenclamide (Jones *et al*., 1996) suggests that BTS 67 582 interacts with either a different site on the sulphonylurea receptor (Ashcroft & Ashcroft, 1992; Malaisse & Lebrun, 1990), or with another receptor (eg. an imidazoline receptor) which is functionally linked to the ATP-sensitive K^+-channel (Morgan *et al*., 1995). It is also possible that BTS 67 582 could promote insulin secretion by a direct interaction with the process of exocytosis that is independent of closure of ATP-sensitive K^+-channels, as recently indicated for sulphonylureas (Eliasson *et al*., 1996).

The present study has shown that the acute hypoglycaemic effect of BTS 67 582 is associated with a marked insulin-releasing effect, which appears to be the main mechanism responsible for the increased glucose utilization.

References

ASHCROFT, S.J.H & ASHCROFT, F.M. (1992). The sulphonylurea receptor. *Biochim. Biophys. Acta*, **1175,** 45–49.

BAILEY, C.J. (1992). Biguanides in NIDDM. *Diabetes Care*, **15,** 755–772.

BAILEY, C.J. (1993). Metformin–an update. *Gen. Pharmacol*, **24,** 1299–1309.

BAILEY, C.J., BROWN, D., SMITH, W. & BONE, A.J. (1987). Xiaoke, a traditional treatment for diabetes. Studies in streptozotocin diabetic mice and spontaneously diabetic BB/E rats. *Diabetes Res*., **4,** 15 – 18.

BAILEY, C.J. & TURNER, R.C. (1996). Metformin. *N. Engl. J. Med*., **334,** 574 – 579.

BAILEY, C.J., WILCOCK, C. & DAY, C. (1992). Effect of metformin on glucose metabolism in the splanchnic bed. *Br. J. Pharmacol*., **105,** 1099 – 1013.

BROWNLEE, M. (1994). Glycation and diabetic complications. *Diabetes*, **43,** 836 – 841.

BYROM, W.D., ROTHERHAM, N.E. & BRATTY, J.R. (1994). Relationship between hypoglycaemic responses and plasma concentration of BTS 67 582 in healthy volunteers. *Br. J. Clin. Pharmacol*., **38,** 433 – 439.

BYROM, W.D., WEIL, A., BROWN, T.J. & BRATTY, J.R. (1996). BTS 67 582 improves 24 h plasma glucose control and post-prandial insulin secretion in NIDDM patients. *Diabetologia*, **39,** A44.

CHERRINGTON, A.D., LACY, W.W. & CHAISSON, J.L. (1978). Effect of glucagon on glucose production during insulin deficiency in the dog. *J. Clin. Invest*., **62,** 664 – 677.

DESBUQUOIS, B. & AURBACH, G.D. (1971). Use of polyethylene glycol to separate free and antibody-bound peptide hormones in radioimmunoassays. *J. Clin. Endocrinol. Metab*., **33,** 732 – 738.

DICKINSON, K., NORTH, T.J. & JONES, R.B. (1997). BTS 67 582 acts as a glucose sensitizing agent in perifused rat pancreatic islets. *Br. J. Pharmacol*., **120,** 189P.

ELIASSON, L., RENSTROM, E., AMMALA, C., BERGGREN, P.O., BERTORELLO, A.M., BOKVIST, K., CHIBALIN, A., DEANEY, J.T., FLATT, P.R., GABEL, J., GROMADA, J., LARSSON, O., LINDSTROM, P., RHODES, C.J. & RORSMAN, P. (1996). PKC-dependent stimulation of exocytosis by sulphonylureas in pancreatic B cells. *Science*, **271,** 813 – 815.

FLATT, P.R. ed. (1992). *Nutrient Regulation of Insulin Secretion*, p415. London: Portland Press.

GROOP, L.C. (1992). Sulfonylureas in NIDDM. *Diabetes Care*, **15,** 737 – 754.

JONES, R.B., DICKINSON, K., ANTHONY, D.M., MARITA, A.R., KAUL, C.L. & BUCKETT, W.R. (1997). Evaluation of BTS 67 582, a novel antidiabetic agent, in normal and diabetic rats. *Br. J. Pharmacol*., **120,** 1135 – 1143.

JONES, R.B., SHEPHERD, R.M., KANE, C., HASHMI, M.N., MOORES, A., HARDING, E.A. & DUNNE, M.J. (1996). Modulation of K-ATP channels and cytosolic Ca^{2+} in isolated insulin-secreting cells by BTS 67 582, a novel hypoglycaemia-inducing agent. *Diabetologia*, **39,** suppl 1, A43.

MALAISSE, W.J. & LEBRUN, P. (1990). Mechanisms of sulfonylurea-induced insulin release. *Diabetes Care*, **13,** suppl.3, 9 – 17.

MORGAN, N.G., CHAN, S.L.F., BROWN, C.A. & TSOLI, E. (1995). Characterization of the imidazoline binding site involved in regulation of insulin secretion. *Ann. New York Acad. Sci. U.S.A*., **763,** 361 – 366.

NOLL, F. (1974). L(+)lactate determination by LDH, GPT and NAD. In *Methods of Enzymatic Analysis*, 2nd edn. ed. Bergmeyer, H.U. pp. 1475 – 1479. New York: Academic Press.

REAVEN, E., WRIGHT, D., MONDON, C.E., SOLOMON, R., HO, H. & REAVEN, G.M. (1983). Effect of age and diet on insulin secretion and insulin action in the rat. *Diabetes*, **32,** 175 – 180.

STEVENS, J.F. (1971). Determination of glucose by an automatic analyser. *Clin. Chim. Acta*., **32,** 199 – 201.

(Received June 18, 1997
Revised September 5, 1997
Accepted September 12, 1997)