J. Pharm. Pharmacol. 1998, 50: 1357–1360
Received March 26, 1998
Accepted July 29, 1998

Biphasic Insulin-releasing Effect of BTS 67 582 in Rats

DAVID A. STOREY AND CLIFFORD J. BAILEY

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

Abstract

BTS 67 582 (1,1-dimethyl-2(2-morpholinophenyl)guanidine fumarate) is being developed as a short-acting anti-diabetic insulin secretagogue. The effect of BTS 67 582 on the phasic pattern of insulin release was assessed in anaesthetized normal rats by measuring arterial plasma insulin concentrations while arterial glucose concentrations were fixed at 6, 8·5 and 12·5 mM.

Intravenous BTS 67 582 ($10\,mg\,kg^{-1}$) induced an immediate but transient increase in insulin concentrations which declined by 10 min (first phase). This was followed by a smaller but sustained increase in insulin concentrations (second phase). The increment from basal to peak insulin release (0–2 min) was independent of glucose, but the first phase was maintained for longer and the second phase was greater at the highest concentration of glucose (12·5 mM). BTS 67 582 also extended the first-phase insulin response to a standard intravenous glucose challenge and enhanced the rate of glucose disappearance by approximately 12%.

Thus BTS 67 582 causes biphasic stimulation of insulin release and augments the insulin-releasing effect of glucose.

The novel anti-diabetic agent BTS 67 582 (1,1-dimethyl-2(2-morpholinophenyl)guanidine fumarate) has been shown to reduce blood glucose concentrations in normal and non-insulin-dependent diabetic animals and man (Byrom et al 1994, 1996; Jones et al 1997; Page & Bailey 1997). This effect is accompanied by increased plasma-insulin concentrations, and a direct insulin-releasing effect was observed when rat isolated islets were perifused with BTS 67 582 (Dickinson et al 1997).

The insulin-releasing drugs currently used to treat non-insulin-dependent diabetes are the sulphonylureas (Gerich 1989). They act directly on pancreatic β-cells, mainly by closing ATP-sensitive potassium (K_{ATP}) channels (Nelson et al 1992). This induces a biphasic pattern of insulin release comprising a rapid, transient burst followed by a smaller but sustained response (Panten et al 1989).

BTS 67 582 also closes K_{ATP} channels (Dunne et al 1995; Jones et al 1996) but seems to act on pancreatic β-cells at a different binding site to sulphonylureas (Jones et al 1997). Thus BTS 67 582 does not displace binding of the sulphonylurea glibenclamide to pancreatic β-cells and exerts some insulin-releasing activity in diabetic animals that are unresponsive to glibenclamide (Jones et al 1997).

This study investigates whether BTS 67 582 induces a biphasic pattern of insulin release similar to that of the sulphonylureas by measurement of arterial plasma insulin concentrations after intravenous drug administration to anaesthetized rats with arterial plasma glucose concentrations fixed at 6, 8·5 and 12·5 mM.

Correspondence: C. J. Bailey, Department of Pharmaceutical and Biological Sciences, Aston University, Aston Triangle, Birmingham B4 7ET, UK.

Materials and Methods

Animals

Adult male Wistar rats, approximately 200 g, were maintained as described elsewhere (Bailey et al 1992). The rats were fasted overnight and anaesthetized with intraperitoneal (i.p.) sodium pentobarbital ($60\,mg\,kg^{-1}$). Anaesthesia was maintained with further doses of sodium pentobarbital ($15\,mg\,kg^{-1}$, i.p.) as required and rectal temperature was maintained at 36°C. Cannulae were introduced into the right jugular vein for administration of drugs and into the left carotid artery for withdrawal of blood samples.

Chemicals and analyses

Chemicals of analytical grade were obtained from BDH (Poole, UK) or Sigma (Poole, UK) except: Sagatal from RMB Animal Health (Dagenham, UK), phosphate-buffered saline (PBS) tablets from Unipath (Basingstoke, UK), and rat insulin standard from Novo Nordisk (Bagsvaerd, Denmark). BTS 67 582 (batch 5 QC1549MM) was supplied by Knoll Pharmaceuticals (Nottingham, UK).

Plasma glucose was measured by means of an automated glucose oxidase procedure (Stevens 1971) using reagents from Beckman (High Wycombe, UK). Plasma insulin was determined by radioimmunoassay using an Amerlex magnetic separation procedure (Amersham Life Science, Amersham, UK) with rat insulin as standard.

Glucose clamps

Arterial plasma glucose concentrations were maintained at 6, 8·5 or 12·5 mM by variable intravenous infusion of a 10% (w/v) glucose solution using a Harvard 22 syringe pump (Harvard Apparatus, Edenbridge, UK). When the required arterial glucose concentration had stabilized for 30 min, either BTS 67 582 ($10\,mg\,kg^{-1}$) or placebo (PBS, $2{\cdot}5\,mL\,kg^{-1}$) was administered as an intravenous bolus, and blood samples (200 μL) were taken 2, 5, 10, 20, 30, 40 and 60 min thereafter for measurement of plasma glucose and insulin. Insulin responses were calculated as the incremental area above basal (time zero) for arterial plasma insulin concentrations at 0–10 min (first phase), 10–60 min (second phase), and 0–60 min (total), calculated by use of the trapezium rule.

Glucose tolerance

Rats with a basal arterial plasma glucose concentration of 6 mM received an intravenous bolus of glucose ($0{\cdot}5\,g\,kg^{-1}$ in 20% w/v solution) with or without BTS 67 582 ($10\,mg\,kg^{-1}$). Blood samples were taken immediately before the injection and 2, 5, 10, 20, 30, 40 and 60 min thereafter for measurement of plasma glucose and insulin. The disappearance of glucose from the plasma was calculated by expressing the value at 30 min as a percentage of the value at 5 min.

Statistics

Data are presented as means ± standard error of the mean (s.e.m.). Incremental insulin responses were compared by analysis of covariance with time zero as a covariate and the treatment–glucose level combination as a factor. Results after administration of BTS 67 582 were compared with control results at the sample glucose clamp level by multiple t (least significant difference) test, and results from the three sets of glucose levels using BTS 67 582 were compared with each other by the Newman–Keuls test. Other results were compared by use of Student's t-test, with $P < 0{\cdot}05$ being accepted as indicative of a significant difference.

Results

Glucose clamps

Intravenous injection of BTS 67 582 ($10\,mg\,kg^{-1}$) elicited a biphasic increase in arterial plasma insulin concentrations at each of the arterial plasma–glucose concentrations studied (Figure 1). Insulin concentrations increased sharply to a peak at approximately 2 min, and declined by 10 min (first phase). This was followed by a sustained increase in insulin concentrations (second phase). The increment in the insulin concentration from basal (0 min) to peak (2 min) was approximately the same at each concentration ($2{\cdot}8 \pm 0{\cdot}4$, $3{\cdot}1 \pm 1{\cdot}1$ and $3{\cdot}0 \pm 0{\cdot}5\,ng\,mL^{-1}$ at 6, 8·5 and 12·5 mM glucose,

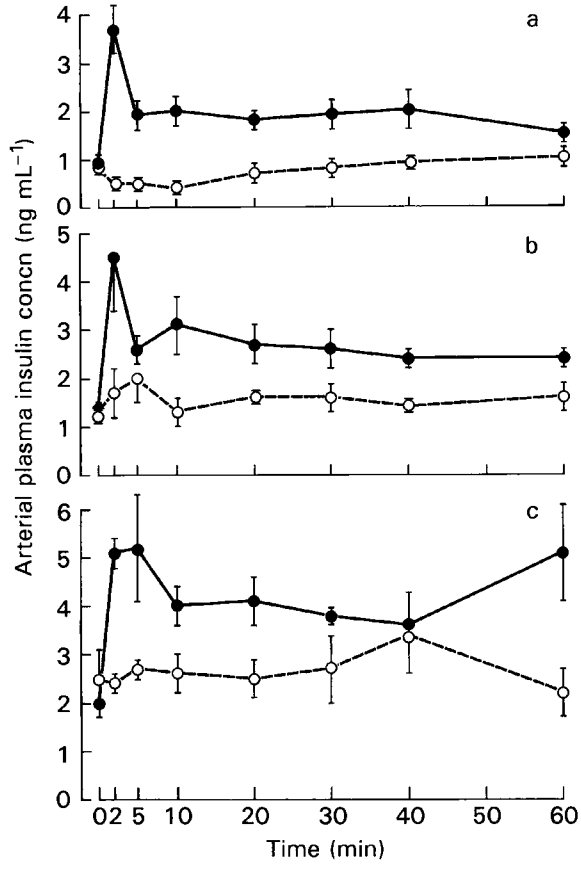

Figure 1. Effect of intravenous BTS 67 582 ($10\,mg\,kg^{-1}$ at 0 min) on arterial plasma insulin concentrations in anaesthetized rats with fixed plasma-glucose levels: a, plasma glucose concentration 6·0 mM; b, plasma glucose concentration 8·5 mM; c, plasma glucose concentration 12·5 mM; ●, BTS 67 582; ○, control. Values are means ± s.e.m. (n = 5–8).

respectively). However, at the highest glucose concentration (12·5 mM) this peak insulin response was more persistent, and the incremental area for the first phase was greater at 12·5 than at 6 mM glucose (Table 1). The incremental area for the second-phase insulin response, and the total incremental area for both phases were also greater at 12·5 mm glucose than at 6 and 8·5 mm glucose (Table 1).

Infusion of glucose was not required to maintain the arterial glucose concentration at 6 mM, presumably because of the efficiency of physiological regulatory mechanisms. Maintaining glucose levels at 8·5 and 12·5 mM required the infusion of increasing amounts of glucose (Table 2). BTS 67 582 increased the amount of glucose required by a similar extent at 8·5 and 12·5 mM glucose (by 33 ± 5 and 28 ± 7 mg glucose h^{-1}, respectively).

Glucose tolerance

As shown in Figure 2, intravenous injection of BTS 67 582 (10 mg kg^{-1}) with an intravenous glucose challenge (0·5 g kg^{-1}) increased the rate of disappearance of glucose from arterial plasma. The percentage decrease in plasma glucose during 5–30 min for the BTS 67 582-treated and control groups was $57·2 \pm 5·0\%$ and $45·3 \pm 2·4\%$, respectively, ($P < 0·05$). The arterial plasma insulin response had a typically biphasic pattern in the control group, whereas BTS 67 582 caused a more protracted first-phase increase in insulin concentrations.

Discussion

In this study arterial plasma insulin concentrations were measured to determine the temporal pattern of insulin release after intravenous bolus administration of BTS 67 582. Glucose levels were fixed to enable determination of the dependency of the

Table 2. Effect of intravenous BTS 67 582 (10 mg kg^{-1}) on the amount of glucose infused to maintain arterial glucose levels at 8·5 and 12·5 mM in anaesthetized rats

	Glucose level (mM)	Amount of glucose infused (mg h^{-1})
Control	8·5	10 ± 5
BTS 67 582	8·5	43 ± 5*
Control	12·5	55 ± 5
BTS 67 582	12·5	83 ± 9*

Values are means ± s.e.m. (n = 5–8). *$P < 0·05$, significantly different from result for control at the same glucose concentration.

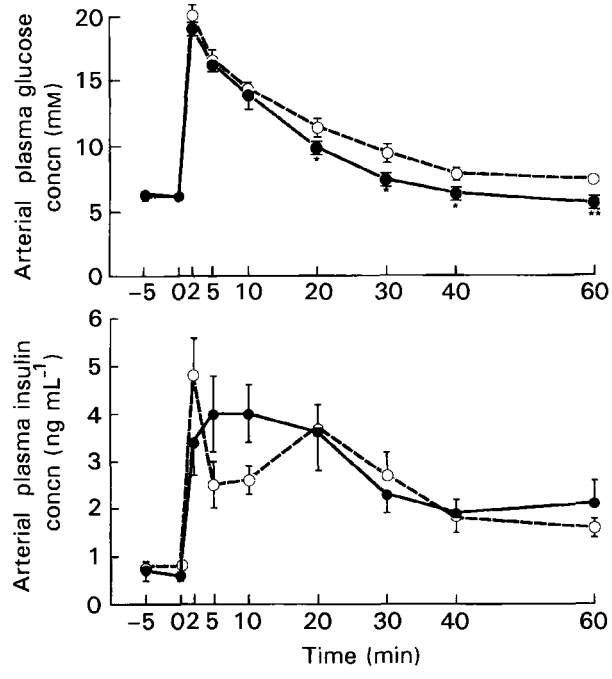

Figure 2. Effect of intravenous BTS 67 582 (10 mg kg^{-1} at 0 min) on arterial plasma glucose and insulin concentrations after an intravenous glucose challenge (0·5 g kg^{-1} at 0 min) in anaesthetized rats: ●, BTS 67 582; ○, control. Values are means ± s.e.m. (n = 6 or 7). *$P < 0·05$, **$P < 0·01$, significantly different from control result.

Table 1. Incremental insulin responses to intravenous BTS 67 582 (10 mg kg^{-1}) in anaesthetized rats with fixed plasma-glucose levels.

	Glucose (mM)	Incremental insulin response (ng)*		Total (0–60 min)
		First phase (0–10 min)	Second phase (10–60 min)	
Control	6	$-3·3 \pm 0·7$	$-9·5 \pm 4·6$	$-12·8 \pm 4·4$
BTS 67 582	6	$13·5 \pm 1·6$	$38·7 \pm 15·4$	$52·1 \pm 16·5$
Control	8·5	$3·9 \pm 1·8$	$7·9 \pm 7·4$	$11·8 \pm 7·8$
BTS 67 582	8·5	$16·4 \pm 4·6$	$56·1 \pm 9·9$	$72·5 \pm 13·9$
Control	12·5	$2·2 \pm 1·4$	$32·4 \pm 21·6$	$34·6 \pm 22·7$
BTS 67 582	12·5	$25·5 \pm 3·7$†	$111·1 \pm 19·5$†‡	$136·6 \pm 20·4$†‡

Values are means ± s.e.m. (n = 5–8). *Incremental insulin response above the basal value (0 min) calculated by use of the trapezium rule. All values for BTS 67 582 were greater ($P < 0·05$) than control values at the same glucose concentration. †$P < 0·05$, significantly different from result for BTS 67 582 with glucose level fixed at 6 mM. ‡$P < 0·05$, significantly different from result for BTS 67 582 with glucose level fixed at 8·5 mM.

insulin responses on glucose concentration, and the dosage of BTS 67 582 (10 mg kg^{-1}) was chosen to reflect a near-maximum insulin-releasing effect based on experience with oral doses up to 300 mg kg^{-1} (Jones et al 1997). BTS 67 582 caused immediate stimulation of insulin release which was independent of arterial glucose concentration in the range 6–12·5 mM. However, the incremental areas for the first and second phases of the insulin response to BTS 67 582 were greater at the highest glucose concentrations.

This pattern of insulin release is similar to that induced by sulphonylurea drugs (Gorus et al 1988; Panten et al 1989). It is consistent with an effect on the pancreatic β-cells that is mediated through closure of K_{ATP} channels, depolarization, and voltage-dependent Ca^{2+} influx, initiating immediate exocytosis of the most labile membrane-associated pool of insulin granules, followed by mobilization of a deeper storage pool (Nelson et al 1992; Grodsky 1994). Because BTS 67 582 augmented insulin release to a greater extent at the highest glucose concentration tested, as seen with sulphonylureas (Basabe et al 1976; Groop 1992), it seems that BTS 67 582 can potentiate glucose-induced insulin release.

Infusion of increased amounts of glucose to maintain the arterial glucose clamps after administration of BTS 67 582 suggests an overall increase in glucose utilization and possibly a decrease in glucose production. This is likely to be almost entirely a result of increased insulin concentrations, because BTS 67 582 did not exert any significant extra-pancreatic glucose-reducing activity in the presence or absence of exogenous insulin in insulin-dependent diabetic BB/S rats (Page & Bailey 1997). Improved disappearance of an intravenous glucose challenge after administration of BTS 67 582 can also be attributed to an extended first-phase insulin response analogous to that seen when the glucose concentration was maintained at the highest level (12·5 mM).

Thus, BTS 67 582 exerts a biphasic stimulatory effect on insulin release similar to sulphonylureas. Although both BTS 67 582 and sulphonylureas cause closure of K_{ATP} channels on pancreatic β-cells, BTS 67 582 binds at a different site to that bound by glibenclamide and has a short duration of action which might offer clinical advantages as an insulin secretagogue which can be taken with a meal to counter postprandial hyperglycaemia (Byrom et al 1994, 1996).

Acknowledgements

The authors thank Dr Richard Brammer for the statistical analyses, and Dr Robert Jones and Dr David Heal for their helpful discussions.

References

Bailey, C. J., Wilcock, C., Day, C. (1992) Effect of metformin on glucose metabolism in the splanchnic bed. Br. J. Pharmacol. 105: 1013–1099

Basabe, J. C., Farina, J. M. S., Chieri, R. A. (1976) Studies on the dynamics and mechanism of glibenclamide-induced insulin secretion. Horm. Metab. Res. 8: 413–419

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 postprandial insulin secretion in NIDDM patients. Diabetologia 39 (Suppl. 1): A44

Dickinson, K., North, T. J., Sills, S., Antony, D. M., Lock, J. I., Vowles, D. T., Jones, R. B. (1997) BTS 67 582 stimulates insulin secretion from perfused rat pancreatic islets. Eur. J. Pharmacol. 339: 69–76

Dunne, M. J., Kane, C., Harding, E. A., Jagger, J. H., Jones, R. B., Ashcroft, S. J. H. (1995) BTS 67 582, a novel insulin-releasing agent. Diabetic Med. 12: S31–S32

Gerich, J. E. (1989) Oral hypoglycemic agents. New Engl. J. Med. 321: 1231–1245

Gorus, F. K., Schuit, F. C., Veld, P. A. I., Gepts, W., Pipeleers, D. G. (1988) Interaction of sulfonylureas with pancreatic β-cells. Diabetes 37: 1090–1095

Grodsky, G. M. (1994) An update on implications of phasic insulin secretion. In: Flatt, P. R., Lenzen, S. (eds) Insulin Secretion and Pancreatic β-cell Research, Smith–Gordon, London, pp 421–430

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

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

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

Nelson, D. A., Aguilar-Bryan, L., Raef, H., Boyd, A. E. (1992) Molecular mechanisms of sulphonylurea action in the pancreatic β-cell. In: Flatt, P. R. (ed.) Nutrient Regulation of Insulin Secretion, Portland Press, London, pp 319–339

Page, T., Bailey, C. J. (1997) Glucose-lowering effect of BTS 67 582. Br. J. Pharmacol. 122: 1464–1468

Panten, U., Burgfeld, J., Goerke, F., Rennicke, M., Schwanstecher, M., Wallasch, A., Zunkler, B. J., Lenzen, S. (1989) Control of insulin secretion by sulphonylureas, meglitinide and diazoxide in relation to their binding to the sulfonylurea receptor in pancreatic islets. Biochem. Pharmacol. 38: 1217–1229

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