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Effect of 1,1-dimethyl-2-[2-morpholinophenyl]guanidine fumarate on pancreatic islet function

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Abstract

The modality of the insulinotropic action of 1,1-dimethyl-2-[2-morpholinophenyl]guanidine fumarate (BTS 67 582), a new antidiabetic agent, was investigated in rat pancreatic islets. At a 0.1 mM concentration, which was sufficient to cause a close-to-maximal secretory response, BTS 67 582 failed to affect the utilization and oxidation of exogenous D-glucose, but slightly augmented $^{14}CO_2$ production from islets prelabelled with either L-[U-^{14}C]glutamine or [U-^{14}C]palmitate. BTS 67 582 (0.1 mM) also failed to affect biosynthetic activity in islets incubated with L-[4-^{3}H]phenylalanine. It augmented insulin release from islets incubated for 90 min in the absence or presence of D-glucose (2.8 to 16.7 mM), this coinciding with stimulation of ^{45}Ca net uptake. In perifused islets deprived of extracellular D-glucose for 45 min, BTS 67 582 (0.1 mM) decreased ^{86}Rb outflow from prelabelled islets, but failed to increase ^{45}Ca efflux and insulin release. In the presence of D-glucose (7.0 mM), BTS 67 582, whilst failing to decrease $^{86}Rb^+$ outflow, provoked rapid, sustained and rapidly reversible increases of both $^{45}Ca^{2+}$ efflux and insulin output. The latter increases were attenuated, but not totally suppressed, in the absence of extracellular Ca^{2+}. BTS 67 582 (0.1 mM) suppressed the inhibitory action of diazoxide (0.25 mM) upon glucose-stimulated insulin release, but nevertheless augmented insulin output from islets incubated in the presence of 90 mM K^+. These findings support the view that the insulinotropic action of BTS 67 582 is mainly attributable to the inactivation of ATP-sensitive K^+ channels. An intracellular redistribution of Ca^{2+} ions may also participate, however, to the islet functional response to BTS 67 582. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: 1,1-Dimethyl-2-[2-morpholinophenyl]guanidine fumarate; Pancreatic islet; Insulin secretion; Cationic flux

1. Introduction

1,1-Dimethyl-2-[2-morpholinophenyl]guanidine fumarate (BTS 67 582) is a novel oral antidiabetic agent recently examined for its effect on glycemia in both healthy volunteers (Byrom et al., 1994) and diabetic patients (Skillman and Raskin, 1997). It was proposed to stimulate insulin release by inhibiting K_{ATP}-channel activity in the pancreatic B-cell, and causing a subsequent elevation of intracellular Ca^{2+} concentration (Dickinson et al., 1997; Jones et al., 1996, 1997). The aim of the present study was to further investigate the effects of this agent upon metabolic, biosynthetic, cationic and secretory variables in isolated rat pancreatic islets.

2. Materials and methods

BTS 67 582 was provided by Knoll Pharmaceuticals (Nottingham, UK).

All experiments were conducted in pancreatic islets isolated by the collagenase procedure (Malaisse-Lagae and Malaisse, 1984) from fed female Wistar rats (Proefdierencentrum, Heverlee, Belgium). The methods used to measure the catabolism of either exogenous nutrients (Malaisse and Sener, 1988) and endogenous nutrients in islets prelabelled with L-[U-^{14}C]glutamine (Malaisse et al., 1981) or [U-^{14}C]palmitate (Sener et al., 1978), the biosynthesis of peptides in islets exposed to L-[4-^{3}H]phenylalanine (Delgado et al., 1991), the secretion of insulin from incubated islets (Malaisse-Lagae and Malaisse, 1984), the net uptake of ^{45}Ca (Malaisse-Lagae and Malaisse, 1971), the outflow of ^{86}Rb (Carpinelli and Malaisse, 1980) and ^{45}Ca (Herchuelz et al., 1980b) and release of insulin (Herchuelz

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Table 1
Effect of BTS 67 582 (0.1 mM) upon the metabolism of D-glucose (8.3 mM)

Metabolic variable	Control	BTS 67 582
D-[5- ^{3}H]glucose utilization[a]	73.80 ± 5.36 (14)	78.14 ± 5.48 (13)
D-[U- ^{14}C]glucose oxidation[a]	33.11 ± 1.63 (16)	34.85 ± 1.54 (15)
D-[U- ^{14}C]glucose conversion to acidic metabolites[a]	22.94 ± 1.64 (16)	26.78 ± 2.56 (15)
D-[U- ^{14}C]glucose conversion to amino acids[a]	13.02 ± 1.51 (16)	14.52 ± 0.82 (16)
D-[U- ^{14}C]glucose oxidation/D-[5- ^{3}H]glucose utilization (%)	45.9 ± 2.7 (14)	46.7 ± 2.4 (13)
D-[U- ^{14}C]glucose/D-[5- ^{3}H]glucose utilization (%)	93.6 ± 3.7 (14)	97.5 ± 4.0 (13)

[a]Results expressed as pmol of glucose equivalent/90 min per islet.

and Malaisse, 1978) from perifused islets (Jijakli et al., 1996) were previously described in the cited references.

All results are presented as mean values (± SEM) together with the number of individual observations (n) or degree of freedom (df). The statistical significance of differences between mean values was assessed by use of Student's t-test.

3. Results

3.1. Metabolic data

As shown in Table 1, BTS 67 582 (0.1 mM) failed to affect significantly the generation of ^{3}HOH from D-[5- ^{3}H]glucose, the oxidation of D-[U-^{14}C]glucose and its conversion to either acidic metabolites or amino acids in islets exposed to a 8.3 mM concentration of the hexose. The paired ratio between D-[U-^{14}C]glucose oxidation and D-[5- ^{3}H]glucose was also comparable in the absence or presence of BTS 67 582. The total recovery of ^{14}C-labelled metabolites generated from D-[U-^{14}C]glucose averaged 95.5 ± 2.7% ($n = 27$) of the mean value for D-[5-^{3}H]glucose utilization.

The effect of BTS 67 582 upon the catabolism of endogenous nutrients was examined in islets preincubated in the presence of either L-[U-^{14}C]glutamine (1.0 mM) or [U-^{14}C]palmitate (0.31 mM).

After 30 min preincubation in the presence of the ^{14}C-labelled amino acid and 8.3 mM D-glucose, the radioactive content of the islets, expressed as L-glutamine equivalent, averaged 12.90 ± 0.44 pmol/islet (Table 2). Over an ensuing incubation of 30 min, conducted in the presence of 8.3 mM D-glucose, the output of $^{14}CO_2$ represented 20.5 ± 0.4% of the initial islet radioactive content, as distinct from only 3.7 ± 0.2% in islets exposed to metabolic poisons (5 mM KCN, 0.01 mM antimycin A and 0.01 mM rotenone). After correction for the latter value, the paired ratio between $^{14}CO_2$ output and islet radioactive content averaged, in the islets exposed to BTS 67 582 (0.1 mM) 108.1 ± 2.7% ($P < 0.05$) of the corresponding mean control value (no BTS 67 582).

After 120 min preincubation in the presence of 8.3 mM D-glucose and ^{14}C-labelled palmitate, the islet radioactive content amounted to 1.80 ± 0.15 pmol/islet, when expressed as palmitate equivalent. The output of $^{14}CO_2$ averaged, during 120 min incubation, at 8.3 mM D-glucose, 6.8 ± 0.4% of the initial radioactive content and was decreased to 1.3 ± 0.1% in the presence of the metabolic poisons. When corrected for the latter measurement, the ratio between $^{14}CO_2$ production and islet radioactive content was again significantly higher ($P < 0.05$) in the presence of BTS 67 582 than in its absence (Table 2).

Table 2
Effect of BTS 67 582 upon the metabolism of endogenous nutrients

Metabolic variable	L-[U-^{14}C]glutamine	[U-^{14}C]palmitate
Islet content (pmol/islet)	12.90 ± 0.44 (99)	1.80 ± 0.15 (98)
$^{14}CO_2$ output	(pmol/islet per 30 min)	(fmol/islet per 120 min)
D-glucose (8.3 mM)	2.30 ± 0.13 (20)	98.4 ± 6.5 (20)
D-glucose (8.3 mM) + BTS 67 582 (0.1 mM)	2.86 ± 0.19 (20)	112.0 ± 6.3 (20)
D-glucose (8.3 mM) + metabolic poisons	0.59 ± 0.05 (20)	19.5 ± 1.7 (20)
$^{14}CO_2$ output/content (%)		
D-glucose (8.3 mM)	20.58 ± 0.38 (20)	6.83 ± 0.42 (18)
D-glucose (8.3 mM) + BTS 67 582 (0.1 mM)	21.95 ± 0.45 (20)	8.18 ± 0.41 (20)
D-glucose (8.3 mM) + metabolic poisons	3.72 ± 0.16 (20)	1.26 ± 0.10 (20)
$^{14}CO_2$ output/content (corrected values in %)		
D-glucose (8.3 mM)	100.0 ± 2.3 (20)	100.0 ± 7.5 (18)
D-glucose (8.3 mM) + BTS 67 582 (0.1 mM)	108.1 ± 2.7 (20)	124.2 ± 7.4 (20)

Table 3
Effect of BTS 67 582 upon biosynthetic activity

D-glucose (mM)	BTS 67 582 (mM)	Trichloroacetic acid-precipitable material (fmol/islet at 90th min)
4.0		241.5 ± 35.7 (14)
4.0	0.1	236.4 ± 47.6 (13)
16.7		604.8 ± 116.3 (14)
16.7	0.1	616.0 ± 123.5 (14)

Taken as a whole, these findings indicate that BTS 67 582, whilst failing to affect the metabolism of exogenous D-glucose, slightly augmented the oxidation of endogenous nutrients.

3.2. Biosynthetic data

When islets were exposed for 90 min to L-[4-^{3}H]phenylalanine (7.6 μM), the incorporation of the tritiated amino acid into trichloroacetic acid-precipitable material increased ($P < 0.01$) as the concentration of D-glucose in the incubation medium was raised from 4.0 to 16.7 mM (Table 3). Whether at low or high hexose concentrations, BTS 67 582 (0.1 mM) failed to affect significantly the labelling of islet peptides.

3.3. Secretory data

D-glucose, at concentrations of 5.6 mM or more, increased ($P < 0.025$ or less), in a concentration-related manner, insulin release above basal value (Table 4, first column; Fig. 1, left panel).

In the absence of D-glucose, 0.1 mM BTS 67 582 significantly augmented insulin secretion, the drug-induced increment in insulin output averaging 8.0 ± 2.5 μU/islet per 90 min ($df = 40$; $P < 0.005$). Likewise, in the presence of only 2.8 mM D-glucose, BTS 67 582, tested at a 1.0 mM concentration, augmented insulin release by 13.2 ± 3.4 μU/islet per 90 min ($df = 42$; $P < 0.001$).

The insulinotropic action of BTS 67 582 was more pronounced ($P < 0.05$) at 5.6 mM D-glucose. Thus, the increment in secretory rate evoked by 0.1 mM BTS 67 582 now averaged 33.1 ± 9.1 μU/islet per 90 min ($df = 40$; $P < 0.001$). Comparable results were recorded in the presence of 7.0 mM D-glucose.

In absolute terms, the increase in insulin release attributable to BTS 67 582 (0.1 mM) was further increased ($P < 0.05$) at 8.3 mM D-glucose relative to that recorded in the presence of 5.6 mM or 7.0 mM D-glucose (Table 4). It indeed reached a mean value of 56.0 ± 8.5 μU/islet per 90 min ($df = 40$; $P < 0.001$) in the presence of 8.3 mM D-glucose, as compared to 32.2 ± 7.5 μU/islet per 90 min ($df = 62$; $P < 0.001$) at the same concentration of BTS 67 582 but in the presence of 5.6–7.0 mM D-glucose.

In the presence of 7.0 mM D-glucose, the concentration–response relationship for the insulinotropic action of BTS 67 582 indicated that as little as 1.0 μM of the drug was sufficient to evoke a significant secretory response ($P < 0.005$) representing about 25% of its maximal value (Fig. 1, right panel).

At the highest concentration of D-glucose tested in the present study (16.7 mM), BTS 67 582 (0.1 to 1.0 mM) also increased insulin release ($P < 0.06$ at 0.1 mM and $P < 0.01$ at 1.0 mM). The absolute value for insulin output was not significantly different at 0.1 and 1.0 mM BTS 67 582. Pooling all measurements, the BTS 67 582-induced increment in secretory rate averaged 34.4 ± 11.6 μU/islet per 90 min ($df = 88$; $P < 0.005$). Such a value was comparable to that found in the presence of 5.6 mM D-glucose.

The release of insulin evoked by 16.7 mM D-glucose was virtually abolished in the presence of 0.25 mM diazoxide (Table 4). In the presence of BTS 67 582 (0.1 mM), however, the glucose-stimulated output of insulin was not significantly affected by diazoxide ($P > 0.15$). BTS 67 582 thus protected the B-cell against the inhibitory action of diazoxide.

The disodium salt of fumarate failed to duplicate the effect of BTS 67 582 upon insulin release from islets incubated in the presence of 7.0 mM D-glucose. The increment in insulin output attributable to fumarate (1.0

Table 4
Effects of D-glucose, diazoxide and BTS 67 582 upon insulin release

D-glucose (mM)	Diazoxide (μM)	BTS 67 582 (mM)			
		Nil	0.01	0.1	1.0
Nil		5.4 ± 2.5 (22)[a]		13.4 ± 2.3 (22)	
2.8		11.0 ± 2.2 (22)			24.2 ± 2.2 (24)
5.6		16.6 ± 3.9 (22)		49.7 ± 8.1 (22)	
7.0		54.3 ± 4.0 (24)	79.4 ± 5.2 (22)	84.8 ± 3.9 (12)	91.4 ± 5.0 (24)
8.3		73.2 ± 7.8 (22)	122.9 ± 8.8 (21)	129.2 ± 8.0 (22)	129.5 ± 8.8 (22)
16.7		215.0 ± 7.7 (48)		239.6 ± 8.7 (24)	259.1 ± 17.1 (24)
16.7	250	17.4 ± 1.7 (24)		222.4 ± 8.1 (24)	

[a] Mean values (± SEM) are expressed as μU/islet per 90 min.

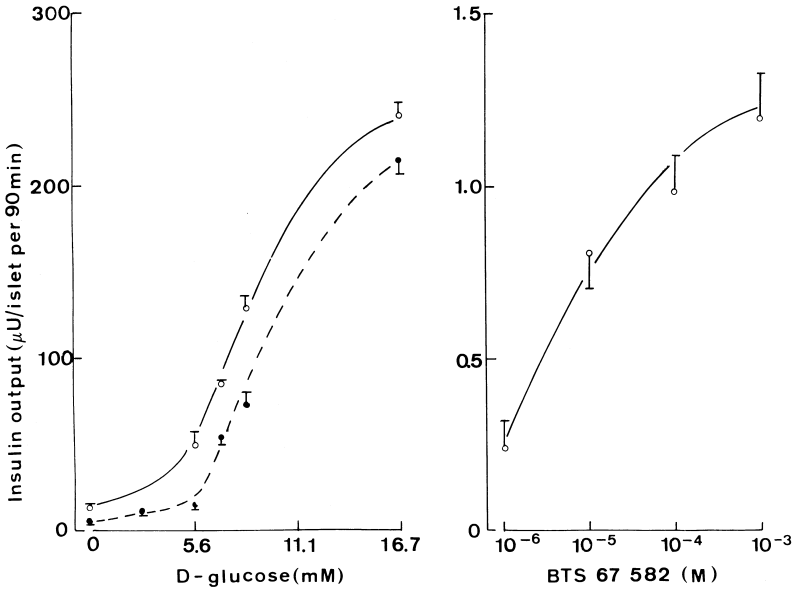

Fig. 1. Left panel: insulin output by islets incubated, at increasing concentrations of D-glucose, in the absence (closed circles and dotted line) or presence (open circles and solid line) of BTS 67 582 (0.1 mM). Mean values (±SEM) refer to 22 to 48 individual observations. Right panel: BTS 67 582-induced increment in insulin release evoked by increasing concentrations of the drug (logarithmic scale) in the presence of 7.0 mM D-glucose. Mean values (±SEM) are expressed relative to the overall average of results recorded, in each experiment, at the three highest concentrations of BTS 67 582, and refer to 24 to 42 individual observations.

mM) averaged 0.3 ± 5.2 μU/islet per 90 min, as compared to 17.8 ± 5.9 ($P < 0.005$) and 60.2 ± 6.5 ($P < 0.001$) μU/islet per 90 min ($df = 56$ in all cases) for that evoked by 1.0 μM and 10.0 μM BTS 67 582, respectively, within the same experiments.

When NaCl (85 mM) was replaced by an equimolar amount of KCl to reach final Na^+ and K^+ concentrations of 54 and 90 mM, respectively, the release of insulin evoked by 7.0 mM D-glucose over 90 min incubation was decreased ($P < 0.001$) by $27.6 \pm 5.9\%$ ($df = 58$) below the control value found at normal Na^+ (139 mM) and K^+ (5 mM) concentrations, as expected from the secondary inactivation of voltage-sensitive Ca^{2+} channels (Plant, 1988). However, BTS 67 582 (0.1 mM) increased to virtually the same extent insulin release from the depolarized islets exposed to a high K^+ concentration or the islets incubated in a salt-balanced medium, the increment in insulin output attributable to BTS 67 582 averaging 24.0 ± 5.2 and 22.4 ± 7.4 μU/islet per 90 min ($df = 58$ and $P < 0.005$ in both cases) in the former and latter situation, respectively.

3.4. Cationic data

After 90 min incubation, the net uptake of $^{45}Ca^{2+}$ was significantly higher ($P < 0.001$; $df = 22$) in islets incubated in the presence of 8.3 mM D-glucose than the basal value (no glucose) measured within the same experiment. BTS 67 582 (0.1 mM) also increased significantly $^{45}Ca^{2+}$ net uptake above basal value ($P < 0.05$; $df = 22$). In the presence of 8.3 mM D-glucose, BTS 67 582 (10 μM to 1.0 mM) caused a concentration-related stimulation of ^{45}Ca net uptake (Table 5). Such an effect achieved statistical significance ($P < 0.05$ or less) except at the lowest concentration of BTS 67 582 (10 μM).

There was a significant correlation ($P < 0.01$) between the mean values for insulin release and ^{45}Ca net uptake (Fig. 2). The increment above basal value for insulin output was not proportional, however, to that for ^{45}Ca uptake, as exemplified by the data recorded in the presence of either 8.3 mM D-glucose or 0.1 mM BTS 67 582.

In order to gain further insight into the mechanism responsible for the BTS 67 582-induced stimulation of $^{45}Ca^{2+}$ net uptake, the efflux of both $^{86}Rb^+$ and $^{45}Ca^{2+}$ from prelabelled islets was monitored in a perifusion design.

In islets perifused in the absence of D-glucose, BTS 67 582 decreased $^{86}Rb^+$ outflow, whether the experiments

Table 5
Effect of BTS 67 582 upon ^{45}Ca net uptake

D-glucose (mM)	BTS 67 582 (mM)	$^{45}Ca^{2+}$ net uptake (pmol/islet at 90th min)
Nil		4.31 ± 0.25 (12)
Nil	0.1	5.51 ± 0.23 (12)
8.3		5.93 ± 0.29 (24)
8.3	0.01	6.36 ± 0.36 (12)
8.3	0.1	6.81 ± 0.28 (24)
8.3	1.0	7.08 ± 0.44 (12)

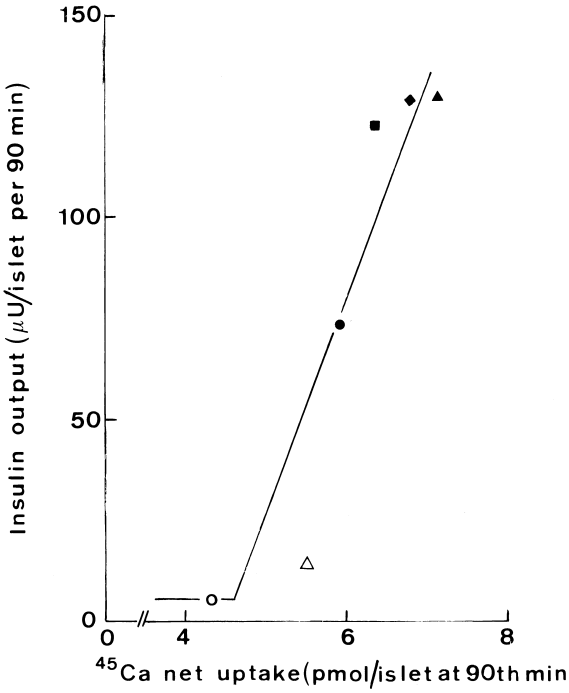

Fig. 2. Relationship between the mean values for insulin release and $^{45}Ca^{2+}$ net uptake in islets incubated without (open symbols) or with (closed symbols) D-glucose (8.3 mM), in the absence (circles) or presence of BTS 67 582 (square: 1.0 μM; diamond: 10.0 μM; triangles: 0.1 mM). The oblique line was calculated by regression analysis.

were conducted at a close-to-normal concentration of extracellular Ca^{2+} (1.0 mM) or in media deprived of $CaCl_2$ and enriched with 0.5 mM EGTA (Fig. 3 Fig. 4, upper left panels). Thus, in both cases, covariance analysis indicated that the slope of the regression line ruling the changes in $^{86}Rb^+$ outflow as a function of time was significantly steeper ($P < 0.025$ or less) over the period of 4–5 min following the introduction of BTS 67 582 than over the preceding 4 to 5 min period. Relative to the paired control value (min 45), the $^{86}Rb^+$ fractional outflow rate at the 5th min of exposure to BTS 67 582 (minute 50) averaged 83.2 ± 2.4 and $84.1 \pm 5.7\%$, respectively in the presence and absence of extracellular Ca^{2+}.

Before introduction of BTS 67 582 (minute 45), the $^{86}Rb^+$ fractional outflow rate was, as expected, lower ($P < 0.05$ or less) in the presence than absence of D-glucose, such being the case at both normal extracellular Ca^{2+} concentration (1.92 ± 0.11 vs. 2.74 ± 0.16 10^{-2} min^{-1}) and in the absence of the divalent cation (1.70 ± 0.04 vs. 2.21 ± 0.18 10^{-2} min^{-1}). Although the $^{86}Rb^+$ fractional outflow rate in islets stimulated by D-glucose tended to be somewhat higher in the presence than absence of extracellular Ca^{2+}, in good agreement with the known effect of the hexose upon Ca^{2+}-responsive K^+ channels, BTS 67 582 caused a minor and transient increase in $^{86}Rb^+$ outflow from glucose-stimulated islets perifused in the presence of Ca^{2+} (Fig. 3, upper right panel), whilst the opposite phenomenon prevailed in the absence of Ca^{2+} (Fig. 4, upper right panel). Thus, 4 min after introduction of BTS 67 582, the $^{86}Rb^+$ fractional outflow rate was 0.13 ± 0.04 10^{-2} min^{-1} higher ($P < 0.05$) than the paired nadir value reached at minute 45 or shortly thereafter in islets perifused in the presence of Ca^{2+}, whilst being 0.16 ± 0.01 10^{-2} min^{-1} lower ($P < 0.001$) than the paired control value (minute 45) in islets perifused in the absence of extracellular Ca^{2+}.

Whether in the presence or absence of extracellular Ca^{2+}, BTS 67 582 (0.1 mM) failed to cause any marked changes in $^{45}Ca^{2+}$ efflux from glucose-deprived islets (Figs. 3 and 4, middle left panels). A transient and minor increase in $^{45}Ca^{2+}$ fractional outflow rate was observed 4–5 min after introduction of BTS 67 582, in the islets perifused in the absence of extracellular Ca^{2+}. Thus, at the 49th–50th min, the $^{45}Ca^{2+}$ fractional outflow rate was 0.18 ± 0.07 10^{-2} min^{-1} higher ($P < 0.05$) than the mean value recorded 3 min earlier. Likewise, at normal Ca^{2+} concentration (1.0 mM), the administration of BTS 67 582 (0.1 mM) transiently slowed down the progressive decrease in $^{45}Ca^{2+}$ fractional outflow rate normally observed during perifusion of the islets. Thus, the slope for the regression line of $\log_e$ fractional outflow rate vs. time averaged -17.2 ± 1.1 and -1.2 ± 2.6 10^{-3} min^{-1} from minute 31 to 45 and from minute 45 to 59, respectively, with a mean paired change of -16.0 ± 3.3 10^{-3} min^{-1} ($n = 4$; $P < 0.02$). Thereafter, however, i.e., from minute 56 to 70, such a slope resumed a significantly negative value (-8.3 ± 1.9 10^{-3} min^{-1}; $P < 0.025$) and then remained virtually unchanged (-11.1 ± 2.7 10^{-3} min^{-1}) after removal of BTS 67 582 from the perifusate (minute 70 to 84).

In the presence of D-glucose (7.0 mM), however, BTS 67 582 provoked a rapid, pronounced, sustained and, at least in part, rapidly reversible increase of $^{45}Ca^{2+}$ efflux (Fig. 3, middle right panel). This stimulation of $^{45}Ca^{2+}$ outflow was decreased, but not abolished, in islets deprived of extracellular Ca^{2+} (Fig. 4, middle right panel). For instance, 5 min after introduction of BTS 67 582, the increment in $^{45}Ca^{2+}$ fractional outflow rate, relative to the paired control value (minute 45) averaged 0.82 ± 0.11 10^{-2} min^{-1} in the presence of extracellular Ca^{2+}, as distinct ($P < 0.01$) from only 0.22 ± 0.12 10^{-2} min^{-1} in its absence.

In the absence of D-glucose and at normal extracellular Ca^{2+} concentration, the administration of BTS 67 582 (0.1 mM) failed to affect significantly insulin release (Fig. 3, lower left panel; Table 6). When these experiments were repeated in the absence of extracellular Ca^{2+}, the output of insulin before introduction of BTS 67 582 was higher ($P < 0.02$) than at normal Ca^{2+} concentration (Table 6), as expected from prior observations (Herchuelz et al., 1980a). In the Ca^{2+}-deprived islets, the paired difference between the integrated release of insulin during the 25 min of exposure to BTS 67 582 and the theoretical value

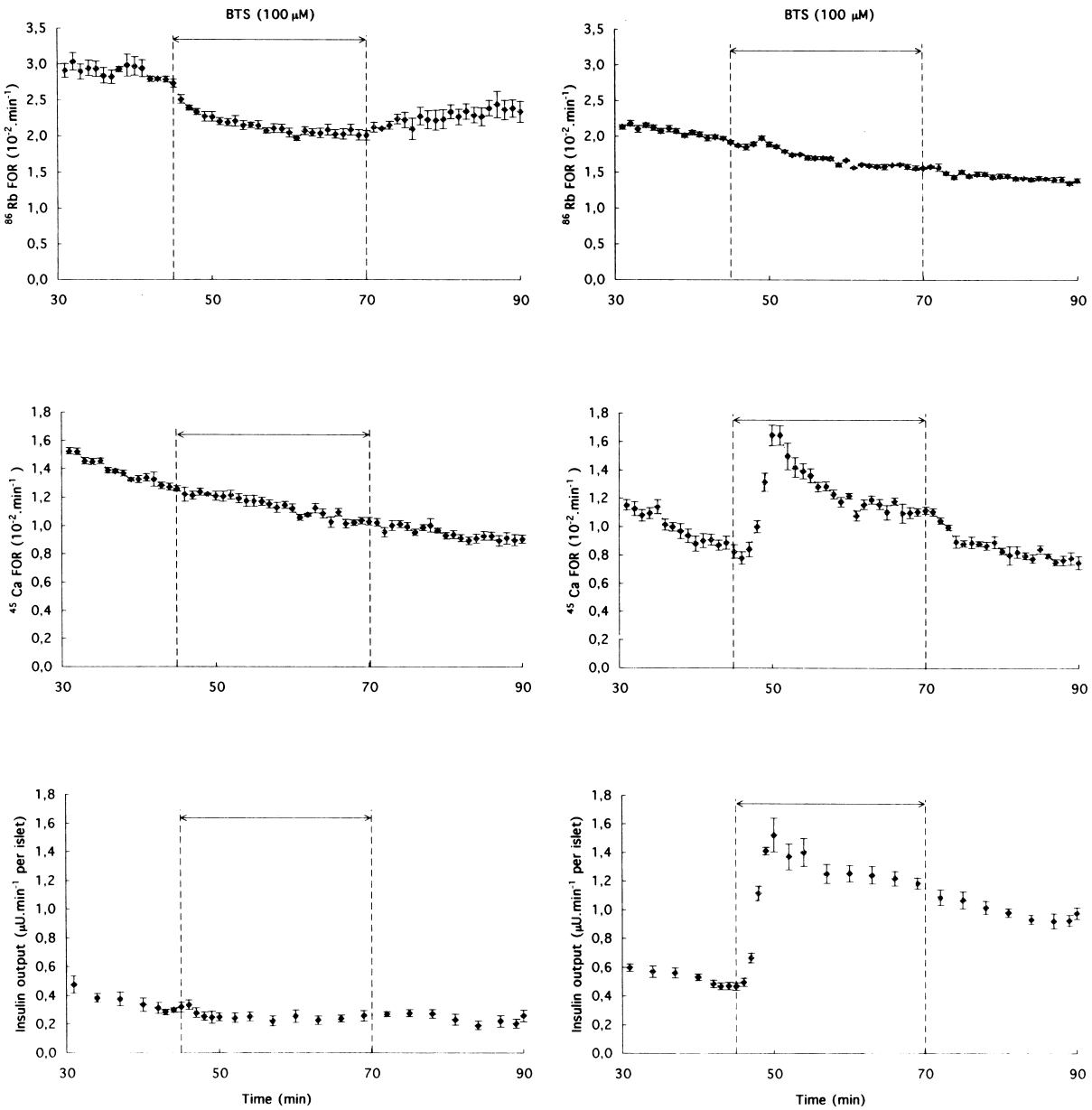

Fig. 3. Effect of BTS 67 582 (0.1 mM) administered from minute 46 to 70 upon $^{86}Rb^{+}$ outflow (upper panels), $^{45}Ca^{2+}$ outflow (middle panels) and insulin release (lower panels) from islets perifused in the absence (left panels) or presence (right panels) of D-glucose (7.0 mM) at normal extracellular Ca^{2+} concentration (1.0 mM). Mean values (±SEM) refer to four (upper panels and middle right panel) or eight (lower panels and middle left panel) individual experiments.

computed by regression analysis of the measurement made before introduction of the antidiabetic agent (minute 42 to 45) and at the end of the experiment (minute 84 to 90) failed to be significantly different from zero (0.15 ± 0.13 μU/min; $n = 8$). However, when the results obtained in each individual experiment were analyzed by the same procedure, a sizeable response to BTS 67 582 was detected in six out of eight cases, the drug-induced increment in insulin output averaging in these six observations $65.6 \pm 12.0\%$ ($P < 0.005$). At normal extracellular Ca^{2+} concentration, the output of insulin before administration of BTS 67 582 was, as expected, higher ($P < 0.01$) in the presence of 7.0 mM D-glucose than in its absence (Table 6). In the presence of the hexose, BTS 67 582 (0.1 mM) provoked a rapid, sustained and not rapidly reversible stimulation of insulin release (Fig. 3, lower right panel). Already within 2 min, the drug caused a paired increase in secretory rate averaging 198 ± 45 nU/min per islet ($P < 0.005$). In the presence of D-glucose (7.0 mM), the output of insulin before introduction of BTS 67 582 was again higher ($P < 0.025$) in the absence than presence of extracellular Ca^{2+} (Table 6). Unexpectedly, in the Ca^{2+}-deprived islets,

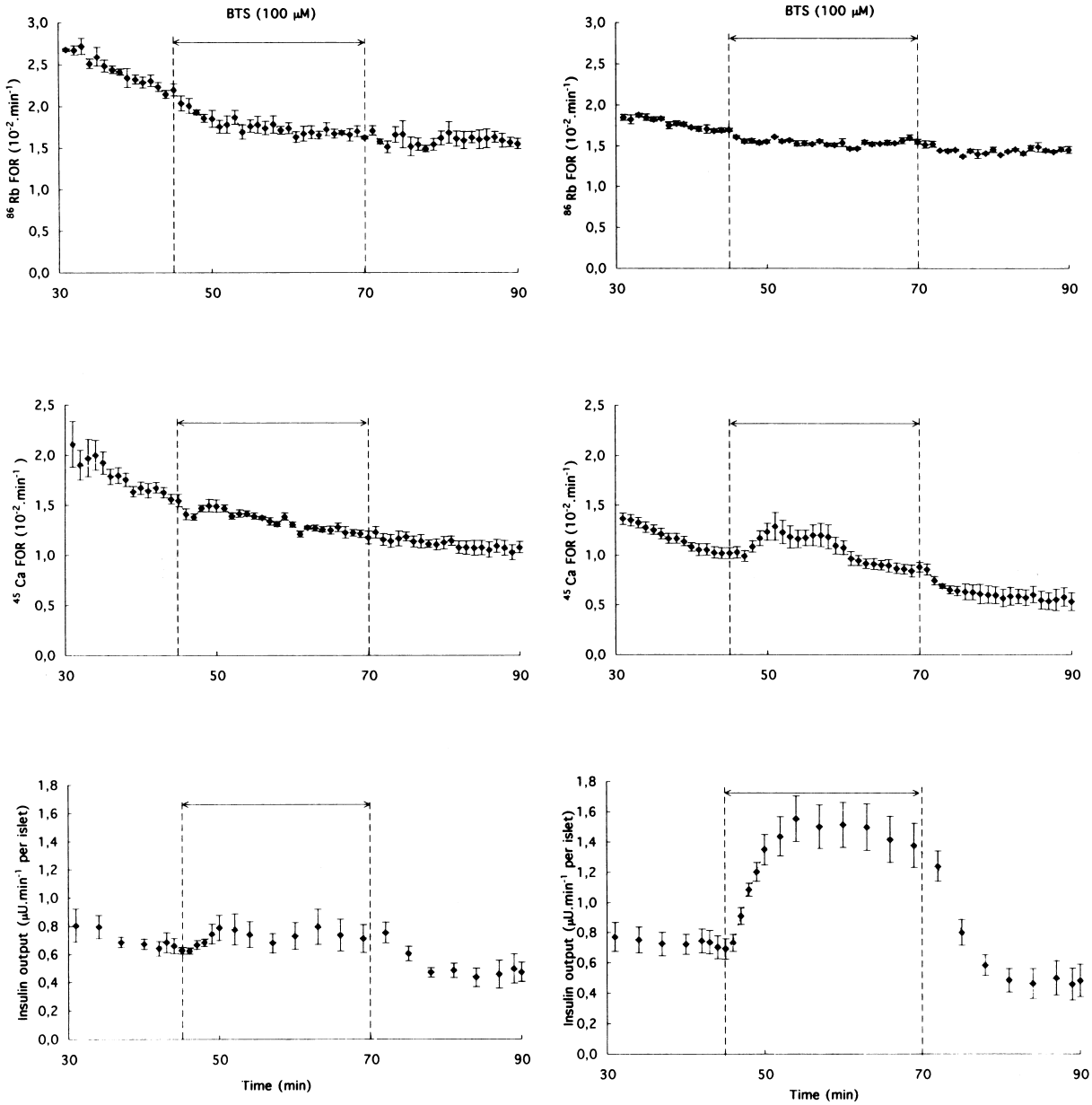

Fig. 4. Effect of BTS 67 582 (0.1 mM) administered from minute 46 to 70 upon $^{86}Rb^{+}$ outflow (upper panels), $^{45}Ca^{2+}$ outflow (middle panels) and insulin release (lower panels) from islets perifused in the absence (left panels) or presence (right panels) of D-glucose (7.0 mM) with media deprived of $CaCl_2$ and containing 0.5 mM EGTA. Mean values (±SEM) refer to four (upper panels and middle left panel), eight (middle right and lower left panels) or twelve (lower right panel) individual experiments.

BTS 67 582 also provoked a rapid, sustained and rapidly reversible increase in insulin secretory rate (Fig. 4, lower right panel). The secretory response to BTS 67 582 was less marked, however, in the absence than presence of Ca^{2+}, at least during the initial period of exposure to the antidiabetic agent. For instance, after 5 min of BTS 67 582 administration, the drug-induced increment in insulin output, relative to the paired value measured at the 45th min represented no more than $97.9 \pm 31.6\%$ ($n = 12$) in the absence of Ca^{2+}, as distinct ($P < 0.02$) from $221.9 \pm 26.1\%$ ($n = 8$) at close-to-normal extracellular Ca^{2+} concentration. Such a difference faded out, however, after prolonged stimulation of the islets by BTS 67 582. Nevertheless, when the delivery of BTS 67 582 was halted at the 70th min of perifusion, the output of insulin from the Ca^{2+}-deprived islets rapidly returned to its initial value, in sharp contrast with the persistent stimulation of insulin release found in the presence of Ca^{2+} after removal of the antidiabetic agent from the perifusate.

In islets incubated for 90 min in the presence of 7.0 mM D-glucose, the increment in insulin output evoked by 0.1 mM BTS 67 582 was not lower in the absence of

Table 6
Effect of BTS 67 582 (0.1 mM) upon insulin release from perifused islets

Ca^{2+} (mM)	D-glucose (mM)	Insulin output (nU/islet per min)		
		minute 42–45	minute 46–70	Paired Δ[a]
1.0	Nil	303 ± 14 (8)	251 ± 23 (8)	−52 ± 35 (8)
Nil	Nil	652 ± 46 (8)	728 ± 75 (8)	+76 ± 119 (8)
1.0	7.0	476 ± 17 (8)	1214 ± 23 (8)	+740 ± 37 (8)
Nil	7.0	720 ± 72 (12)	1371 ± 108 (12)	+651 ± 179 (12)

[a]Paired BTS 67 582-induced change in insulin release, as judged from the mean secretory rates before (minute 42–45) and during (minute 46–70) administration of the drug.

extracellular Ca^{2+} and presence of EGTA (0.5 mM) than at normal Ca^{2+} concentration (1.0 mM), with mean respective values of 67.6 ± 12.5 and 59.2 ± 13.4 μU/islet per 90 min ($df = 28$ in both cases).

4. Discussion

Several of the present findings are consistent with the idea that BTS 67 582 acts upon islet B-cells by a sequence of events similar to that currently implied in the insulinotropic action of hypoglycemic sulfonylureas or meglitinide analogues, i.e., a decrease in K^+ conductance leading to depolarization of the plasma membrane, gating of voltage-sensitive Ca^{2+} channels and eventual activation by the divalent cation of the effector system for translocation and exocytosis of insulin secretory granules (Jones et al., 1996).

First, BTS 67 582 indeed decreased $^{86}Rb^+$ outflow from prelabelled islets perifused in the absence of any exogenous nutrient. This effect did not appear attributable to any marked change in the catabolism of either endogenous or exogenous nutrients. At variance with the process of nutrient-induced insulin release, the insulinotropic action of BTS 67 582 also failed to be associated with enhanced biosynthesis of islet peptides. As expected from prior studies conducted with other secretagogues (Carpinelli and Malaisse, 1981; Lebrun and Malaisse, 1992; Malaisse and Sato, 1995), the inhibitory action of BTS 67 582 upon $^{86}Rb^+$ outflow was only masked in islets already exposed to a stimulatory concentration of D-glucose and perifused in the presence of extracellular Ca^{2+}.

Second, BTS 67 582 indeed increased $^{45}Ca^{2+}$ net uptake. This appeared to be attributable, in part at least, to stimulation of Ca^{2+} influx into the islet cells. Indeed, in islets perifused in the presence of 7.0 mM D-glucose and at normal extracellular Ca^{2+} concentration, BTS 67 582 markedly augmented $^{45}Ca^{2+}$ efflux from prelabelled islets, such an effect being much less pronounced in the absence of extracellular Ca^{2+}. These findings are thus consistent with an exchange process between influent $^{40}Ca^{2+}$ and effluent $^{45}Ca^{2+}$. The relationship between $^{45}Ca^{2+}$ net uptake and insulin release was also reminiscent of that found with other insulinotropic antidiabetic agents (Hubinont et al., 1986; Malaisse et al., 1972).

Last, as it is the case with hypoglycemic sulfonylureas or meglitinide analogues, BTS 67 582 protected the B-cells against the inhibitory action of diazoxide upon glucose-stimulated insulin release.

Three of the present findings differ, however, from those observed, under identical experimental conditions, in islets exposed to either hypoglycemic sulfonylureas or meglitinide analogues. First, in the absence of D-glucose, the inhibition of $^{86}Rb^+$ outflow by BTS 67 582, even when tested at an 0.1 mM concentration, was less pronounced than that caused by the other antidiabetic agents (Lebrun and Malaisse, 1992; Malaisse and Sato, 1995). Second, in the absence of D-glucose and presence of extracellular Ca^{2+}, BTS 67 582 virtually failed to enhance $^{45}Ca^{2+}$ efflux. Third, in the presence of 7.0 mM D-glucose, the insulinotropic action of BTS 67 582 was not suppressed in incubated or perifused islets deprived of extracellular Ca^{2+}. Even in the absence of D-glucose, BTS 67 582 caused, in six out of eight individual experiments, a sizeable increase in insulin output from islets perifused in the absence of extracellular Ca^{2+}.

These three unexpected findings could well be interrelated. Indeed, the relatively modest effect of BTS 67 582 upon $^{86}Rb^+$ outflow may account for its failure to stimulate Ca^{2+} entry and insulin release in islets that were deprived of extracellular nutrient both for 45 min before introduction of BTS 67 582 and during subsequent exposure to this agent. Under these conditions, the BTS 67 582-induced inhibition of K^+ conductance may not be sufficient to reach the threshold value required for the gating of voltage-sensitive Ca^{2+} channels (Carpinelli and Malaisse, 1981). A somewhat different situation prevailed when the islets were immediately exposed to BTS 67 582 (0.1 mM) after isolation, in which case a modest but significant increase in insulin output above basal value was recorded over 90 min incubation.

The relatively weak effect of BTS 67 582 on K^+ conductance could in turn reflect an intracellular redistribution of Ca^{2+} ions. Such a hypothesis is supported by the following observations. In Ca^{2+}-deprived islets, BTS 67 582 increased $^{45}Ca^{2+}$ outflow, this effect being most marked in the presence of D-glucose, which favours the

retention of $^{45}Ca^{2+}$ in the microsomal compartment (Manuel y Keenoy et al., 1990). Second, an increase in cytosolic Ca^{2+} concentration, possibly resulting from the postulated intracellular redistribution of the divalent cation, could indeed antagonize the inhibitory action of BTS 67 582 on $^{86}Rb^{+}$ outflow, as otherwise attributable to the closing of ATP-sensitive K^{+} channels. The intracellular translocation of Ca^{2+} might also account for the persistence of a sizeable secretory response to BTS 67 582 in islets perifused in the absence or presence of D-glucose but absence of extracellular Ca^{2+} and in islets incubated at a high concentration of extracellular K^{+} (90 mM). Last, an increase in cytosolic Ca^{2+} activity, with resulting enhanced mitochondrial net uptake of the divalent cation, may favour mitochondrial oxidative processes (Malaisse, 1992), this possibly accounting for the modest but significant increase in $^{14}CO_2$ output from islets prelabelled with either [U-^{14}C]palmitate or L-[U-^{14}C]glutamine.

In conclusion, therefore, the mode of action of BTS 67 582 in the islet B-cells seems to differ in certain respects, from that of other agents used as insulinotropic tools for the treatment of non-insulin-dependent diabetes. Indeed, the insulinotropic action of BTS 67 582, although appearing mainly attributable to a primary decrease in K^{+} conductance at the level of the plasma membrane, may also entail an intracellular redistribution of Ca^{2+}. The BTS 67 582 target system involved in the latter phenomenon remains to be identified.

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