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Research Article

Bromocriptine/SKF38393 ameliorates islet dysfunction in the diabetic (*db*/*db*) mouse

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Abstract. Dysfunction of pancreatic islets plays a crucial role in the etiology of type II diabetes. Chronic hyperglycaemia or hyperlipidaemia may impair islet function. Previous studies by our laboratory have demonstrated that dopaminergic agonists ameliorated hyperglycaemia and hyperlipidaemia in obese and diabetic rodents. In the present study, we investigated the effect of a treatment with the dopamine D_2/D_1 receptor agonists (bromocriptine/SKF38393, BC/SKF) on islet dysfunction in *db*/*db* mice. Our results show that a 2-week BC/SKF treatment markedly reduced hyperglycaemia and hyperlipidaemia, and significantly improved islet dysfunction demonstrated by an increase of secretagogue-stimulated insulin release from islets of *db*/*db* mice to levels observed in islets from lean mice. There was also a fourfold increase of insulin content in the pancreas of BC/SKF-treated *db*/*db* mice compared with that in untreated controls. The effect of BC/SKF on islet function cannot be mimicked in pair-fed animals. BC/SKF had no direct stimulatory effect on islet insulin secretion, suggesting BC/SKF treatment improved islet function via an indirect mechanism. This treatment markedly improved the abnormally elevated daily levels of corticosterone, blood glucose and plasma lipids, supporting the view that BC/SKF may affect the neuroendocrine system that in turn regulates peripheral metabolism and thereby improves islet function.

Key words. Insulin release; pancreatic islet; diabetic animal model; dopaminergic agonist.

Insulin release from the pancreatic β cells is regulated by many factors that include hormones, paracrine activities and autonomic neural activities, as well as blood-borne nutrients such as glucose, fatty acids and amino acids. Disorders in these parameters may alter normal insulin release, and islet dysfunction is an important causal factor in the pathogenesis of type II diabetes. However, the means by which islet dysfunction is initiated are unclear. It has been proposed that chronic hyperglycaemia or hyperlipidaemia resulting from peripheral insulin resistance may impair secretagogue-induced insulin release [1–4]. If so, a reduction of blood glucose and lipid levels in type II diabetic subjects may benefit pancreatic islet function.

Neuroendocrine activities have important influences on glucose and lipid metabolism, and dopaminergic ago-

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nists provided at certain times of day alter the daily profiles of several hormones and influence peripheral energy metabolism to reduce body fat stores and improve insulin sensitivity [5]. Our laboratory previously demonstrated that systemic administration of dopamine receptor agonists dramatically reduced blood glucose and free fatty acid (FFA) levels in the seasonal obese-insulin resistant hamster [6], the genetically obese *ob*/*ob* mouse [7] and overweight humans [8]. These effects are associated with reductions in norepinephrine and serotonin activities in the ventromedial hypothalamus (VMH) [9], high levels of which potentiate the hyperglycaemic and hyperlipidaemic state [10, 11]. These findings suggest that dopaminergic agonists may act through the neuroendocrine system to improve peripheral energy metabolism. Consequently, dopaminergic agonists may also improve impaired islet function in type II diabetes by impacting hypothalamic centres, as well as reducing chronic hyperglycaemia and hyperlipidaemia.

Therefore, in the present study, we investigated the effect of a combination treatment with dopamine D_1 and D_2 receptor agonists (SKF 38393 and bromocriptine) on islet function in the *db*/*db* mouse. The C57BL/KsJ-*db*/*db* mouse is a diabetic animal model characterized by obesity, hyperglycaemia, temporarily elevated plasma insulin and progressive degenerative changes in the islets of Langerhans which results in lethal diabetes [12–14]. Our results show that BC/SKF treatment of *db*/*db* mice (i) significantly reduced hyperglycaemia and hyperlipidaemia and enhanced insulin release in vivo; and (ii) improved secretagogue-stimulated insulin release tested in vitro to levels equivalent to those of normal mice.

Methods

Animal treatments and sample collections. C57BL/KsJ *db*/*db* mice and their lean littermates (C57BL/KsJ +/+) were used in this study. Mice were 6–7 weeks of age and housed two per cage on a 12/12 light-dark cycle environment and fed rodent chow (PMI Feeds, St. Louis, MO, USA) ad libitum. We demonstrated previously that combination treatment of hyperglycaemic *ob*/*ob* mice with bromocriptine plus SKF38393 produced synergistic effects to reduce hyperglycaemia and hyperlipidaemia [7]. In the present study, BC/SKF or vehicle was injected i.p. at 1 h after light onset (HALO) for 14 days. The daily doses of BC and SKF were 16 and 20 mg/kg body weight, respectively. These doses are based on previous studies in rodents that demonstrated central effects from systemic administration of BC and SKF [7, 15]. Body weight was monitored weekly, and food consumption was measured daily. A group of vehicle-injected *db*/*db* mice was pair fed on a daily basis with the same amount of food as that consumed by the BC/SKF treated group. In the first study (experiment 1), mice were sacrificed at 4 HALO on the 15th day of the study, to collect blood for biochemical analyses. Pancreatic tissue was then removed for islet morphology studies or for islet isolations used for insulin release study in vitro. In the second study (experiment 2), *db*/*db* mice and their littermates were treated as above and then sacrificed at 0, 4, 8, 12, 16 or 20 HALO 1 day after 14 days of treatment. Blood samples were collected for analyses of daily rhythms of serum corticosterone, insulin, glucose and lipids.

Histology and immunocytochemistry. The gastrosplenic portion of the pancreas from *db*/*db* mice (control and treated) and their lean littermates was immersion-fixed in 4.0% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS; 100 mM phosphate salts + 150 mM NaCl, pH 7.6) for 3 h on ice. Following overnight washing in PBS, pancreata were dehydrated in ascending ethanols and embedded in paraffin by conventional methods. Five-micron sections were stained with haematoxylin and eosin (H&E) or immunostained for insulin and glucagon to mark islet β and α cells, respectively. Double-immunofluorescent staining was accomplished by initially blocking sections with 5% normal donkey serum + 1.0% bovine serum albumin (BSA) in 10 mM PBS followed by overnight incubation in 1:2000 dilution of guinea pig anti-insulin serum and rabbit antiglucagon serum (Linco, St. Charles, MO, USA) in dilution buffer (PBS + 1.0% BSA + 0.1% Triton X-100). Following washing sections 3 × 15 min, sections were incubated in 1:250 dilution donkey antiguinea pig IgG-fluorescein isothiocyanate (FITC) and 1:2000 donkey anti-rabbit IgG-CY3 ('ML' grade, Jackson Immunoresearch, West Grove, PA, USA). After washing and mounting in Aqua-Polymount (Polysciences, Warrington, PA, USA), sections were imaged under identical conditions on a Zeiss LSM 410 laser-scanning confocal microscope (Cell Imaging Core Facility, Vanderbilt School of Medicine). Digital images of both H&E and immunofluorescent-stained pancreata were assembled on a Silicon Graphics Indigo workstation and printed on a Tektronix dye-sublimation color printer.

Islet insulin release study. Insulin release induced by various secretagogues was studied using static islet incubation [16]. Pancreatic islets were isolated by collagenase digestion and collected under the dissection microscope. A group of 10 islets was loaded in each incubation tube. After 20 min preincubation, islets were transferred to incubation buffer (Hepes-buffered Krebs-Ringer medium, pH 7.4) containing one of several secretagogues described in the 'Results'. Both the preincubation and incubation were performed at 37 °C in a

Table 1. The effect of BC/SKF treatment on *db*/*db* mice.

	Body weight gain in 14 days (g)	Food intake (g/day)	Blood glucose (mmol/l)	Plasma insulin (pmol/l)	Plasma FFA (mmol/l)
Lean mouse, ad libitum	1.3 ± 0.2	3.1 ± 0.1	8.5 ± 0.3	233 ± 52	0.97 ± 0.08¶
Lean mouse (BC/SKF), ad libitum	2.0 ± 0.8	3.0 ± 0.1	8.2 ± 0.3	316 ± 83	$0.57 \pm 0.03^{\&\&}$
db/*db* (vehicle-injected), ad libitum	$4.4 \pm 0.6^{\&\&}$	$8.4 \pm 0.7^{\&\&}$	$33.6 \pm 1.7^{\&\&}$	$2{,}650 \pm 367^{\&\&}$	1.09 ± 0.11
db/*db* (vehicle-injected), pair-fed	1.6 ± 0.6**	$6.4 \pm 0.2^{\&\&,**}$	$20.5 \pm 1.9^{\&\&,**}$	$3{,}083 \pm 517^{\&\&}$	$1.29 \pm 0.08^{\&\&}$
db/*db* (BC/SKF), ad libitum	$5.0 \pm 0.4^{\&\&,\#\#}$	$6.3 \pm 0.1^{\&\&,**}$	$19.2 \pm 1.6^{\&\&,**}$	$9{,}517 \pm 1{,}167^{\&\&,**,\#\#}$	$0.58 \pm 0.05^{\&\&,*,\#\#}$

Diabetic *db*/*db* mice and their lean controls were treated with BC/SKF or vehicle for 14 days. Food intake was monitored daily, and body weight was measured at days 0 and 15 of treatment to obtain the delta. Mice were decapitated at 4 HALO, and trunk blood was collected to determine the blood glucose, plasma insulin and FFA levels.
$^{\&\&}P < 0.01$, compared with lean mice. $^{*}P < 0.05$, compared with vehicle-injected *db*/*db* mice. $^{**}P < 0.01$, compared with vehicle-injected *db*/*db* mice. $^{\#\#}P < 0.01$, compared with pair-fed *db*/*db* mice (ANOVA). ¶The plasma FFA level in C57BL/KsJ +/+ mice ranges from 0.5 to 0.9 mM. The lean mice we used in this study showed a higher mean value of plasma FFA. This may be due to the variation in lean mice from different purchases (P. W. D. Scislowski, unpublished data).

water bath shaker with an atmosphere of 95%O_2/5%CO_2. Samples of incubation buffer were collected at 0 and 60 min to measure insulin content by Radioimmunoassay (RIA, Linco, St. Charles, MI, USA).

Islet DNA and pancreatic insulin content determinations. The DNA content in isolated islets was determined by a fluorometric method using a DyNA Quant 200 Fluorometer (Hoefer Pharmacia Biotech, San Francisco, CA, USA). Insulin content in the pancreatic tissue was measured using the supernate of the acid-ethanol extraction from pancreas followed by RIA.

Determinations of metabolites and hormones in the blood. Blood glucose was measured by a glucose monitor (Accu-Check Advantage, Boehringer, Indianapolis, IN, USA) and a spectrophotometric method using glucose oxidase coupled to peroxidase (Sigma Diagnostics, St. Louis, MO, USA). Plasma FFA was determined by enzymatic assay utilizing acyl-coenzyme (CoA) synthetase and acyl-CoA oxidase coupled to peroxidase (Wako Chemicals USA, Richmond, VA, USA). Triglyceride concentration in the plasma was determined enzymatically utilizing lipoprotein lipase coupled to glycerol kinase (Sigma Diagnostics). Commercially available RIA assay kits were utilized to measure the plasma concentrations of insulin (Linco Research) and corticosterone (ICN Pharmaceuticals, Costa Mesa, CA, USA).

Statistical analyses. Statistical analyses of differences in measured parameters between groups were performed with two-way analysis of variance (ANOVA).

Results

Experiment 1: effects of BC/SKF treatment on the diabetic syndrome and islet function in the *db*/*db* mouse. After a 14-day treatment of BC/SKF, blood glucose and plasma FFA levels in *db*/*db* mice were reduced by 43 and 47%, respectively, compared with those in the vehicle-injected *db*/*db* control. Furthermore, plasma insulin concentration was threefold greater in the BC/SKF-treated group than in *db*/*db* controls. Daily food consumption was significantly reduced in the treated mice, but body weight was unchanged. In the pair-fed *db*/*db* mice, blood glucose was also reduced to a level similar to that in the BC/SKF-treated mice; however, no enhancement of plasma insulin or reduction of plasma FFA was found in these pair-fed *db*/*db* mice. We also treated the lean mice with BC/SKF in the same manner and found that, other than a 40% decrease in the plasma FFA level, there were no differences regarding blood glucose, insulin and food intake between the lean control and treated groups (table 1).

The morphology study showed that islets from *db*/*db* mice were generally larger and more numerous compared with those of lean mice (fig. 1A,C,E). Insulin-producing β cells of vehicle-injected and BC/SKF-treated *db*/*db* mice (fig. 1C,E) were both moderately hypertrophied with increased cytoplasmic eosinophilia compared with islets from lean mice (fig. 1A). Insulin immunofluorescence revealed fairly uniform staining of β cells from lean animals (fig. 1B), but a moderate to strongly heterogeneous staining pattern was observed in islets from both the untreated and treated *db*/*db* mice (fig. 1D, F). Qualitative analysis showed that, although vehicle-injected *db*/*db* animals exhibited islets with generally lower insulin immunofluorescence (as shown by a less bright green colour, fig. 1D) compared with lean mice (fig. 1B). Treated *db*/*db* mice displayed islets with enhanced insulin staining approximating the intensity of those from lean control mice (fig. 1B, F, respectively).

The insulin content in pancreatic tissue was measured to further evaluate the effect of BC/SKF treatment. In lean mice, the insulin content in pancreas was 7.4 ± 1.0 nmol/g wet tissue. This value was 3.2 ± 0.7 nmol/g wet

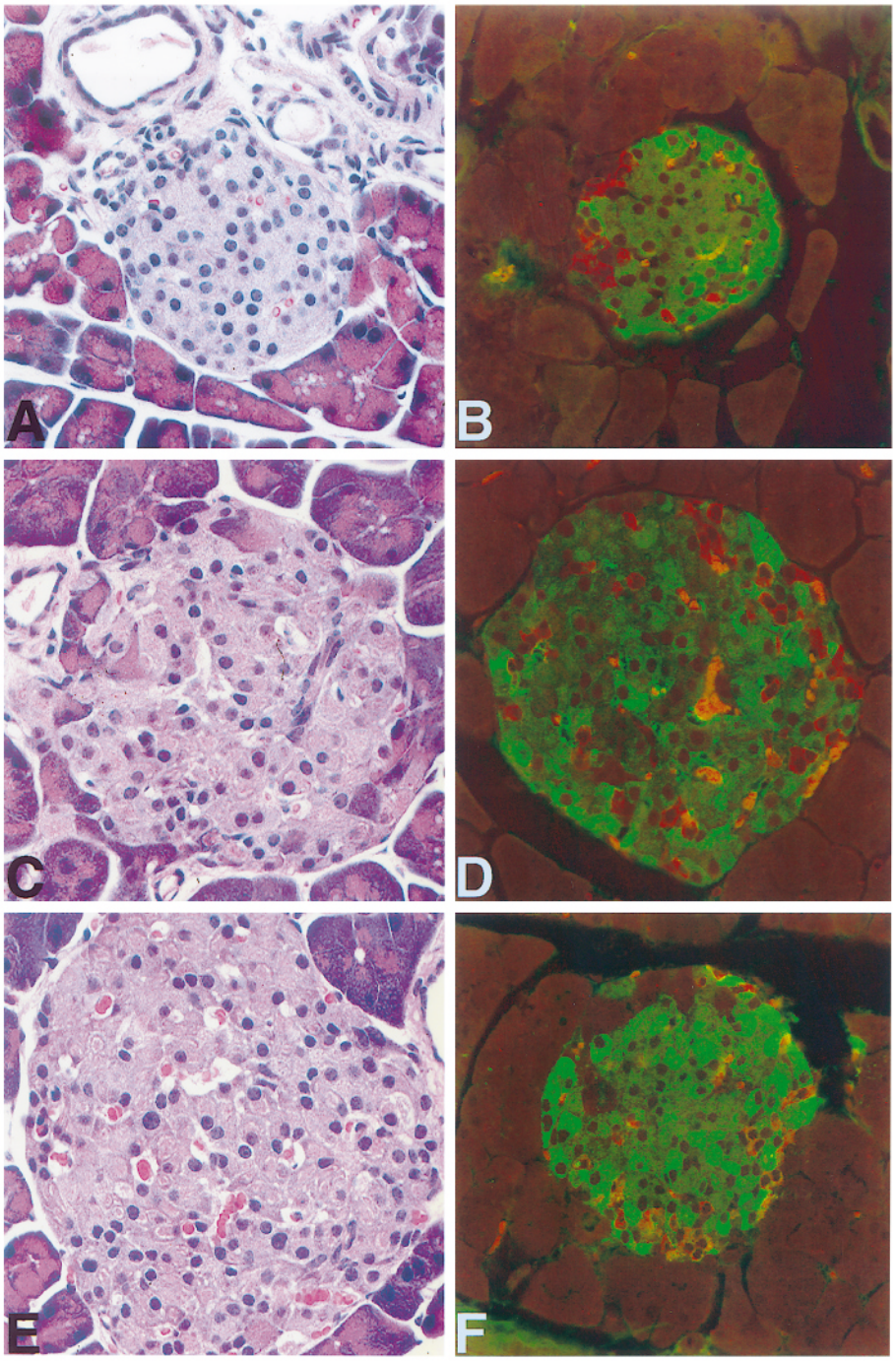

Figure 1. Histology and immunocytochemistry of islets from lean, *db*/*db* control and *db*/*db* treated mice. *A*, *C* and *E* are H&E stained. *B*, *D* and *F* are immunostained for insulin (green) and glucagon (red). (*A* and *B*) Representative islet from lean mouse (C57BL/KsJ +/+). Double-immunofluorescence reveals nearly uniform insulin staining (green) of β cells in islet core. Enhanced green fluorescence indicates increased levels of insulin. Peripheral glucagon-producing α cells (red) and erythrocytes (orange) are also observed. (*C* and *D*) Representative islet from *db*/*db* control (vehicle-injected) mouse. Insulin immunofluorescence shows low-to-moderate signal with a nonuniform pattern of staining of β cells. (*E* and *F*) Representative islet from treated *db*/*db* mouse. (*F*) Insulin immunofluorescence pattern reveals moderate to intense, but nonuniform, pattern of insulin staining of β cells. Width of each field is approximately 150 mm.

tissue in the vehicle-injected *db/db* mice, regardless of the increased total islet volume in the pancreas. However, the insulin content was increased by fourfold in BC/SKF-treated *db/db* mice (13.7 ± 3.1 nmol per gram of wet tissue, $P < 0.01$ compared with vehicle-injected *db/db* mice). Considering the enhanced plasma insulin level in BC/SKF-treated *db/db* mice, increased islet insulin content is most likely not due to a reduced secretory activity but rather suggests an increased insulin biosynthesis in these mice. DNA content in isolated islets from untreated *db/db* mice was significantly higher than that in the lean mice (42.6 ± 6.1 vs. 23.4 ± 3.6 ng/islet, $P < 0.05$). BC/SKF treatment did not change the islet DNA content of *db/db* mice (42.6 ± 6.1 and 45.2 ± 5.8 ng/islet for *db/db* control and treated groups, respectively).

Insulin release induced by secretagogues was studied using static islet incubation. Due to hypertrophy and hyperplasia of pancreatic islets [13], *db/db* mice showed 10-fold higher plasma insulin levels compared with lean mice. However, the glucose-induced insulin release in vitro normalized per islet DNA content was significantly reduced in *db/db* mice compared with that from lean mice (fig. 2), suggesting a pancreatic β-cell dysfunction and/or exhaustion. After 14 days of BC/SKF treatment, insulin release from islets incubated with medium containing 8 or 15 mM glucose was significantly enhanced (two- to fourfold) compared with that in the vehicle-injected *db/db* mice (fig. 2A). Insulin release in response to nonglucose secretagogues (arginine, glucagon-like peptide and acetylcholine) was enhanced two- to threefold in the islets of treated *db/db* mice compared with that in the vehicle-injected *db/db* controls (fig. 2B). We used islet DNA content to standardize the insulin release from pancreatic islets of different sizes and found that the secretagogue-induced insulin release from islets in BC/SKF-treated mice reached the same level as that in lean controls. This indicated that insulin release of islets from *db/db* mice was significantly improved by BC/SKF treatment to the level of islets from lean mice. Pair-feeding *db/db* mice had no effect on secretagogue-stimulated insulin release. We also tested the insulin release from islets of lean mice treated with BC/SKF and found that there were no significant differences in insulin release between the control and treated lean mice (fig. 2A, B). Moreover, similar responses were obtained even with older *db/db* mice (3 months of age) but to a lesser extent (50% improvement; data not shown).

To test whether BC/SKF had a direct stimulatory effect on pancreatic islets, BC, SKF or a combination thereof was directly added to the buffer for static incubation using islets isolated from untreated *db/db* mice (fig. 3A,B). Two glucose concentrations (8 and 15 mM) were applied in this study. Islet insulin release was not significantly affected by exposure to BC or SKF alone. However, BC/SKF at 1 μM but not at 1 nM in the incubation medium reduced insulin release ($P < 0.05$) at 15 mM glucose only.

Experiment 2: effects of BC/SKF treatment on daily rhythms of corticosterone, insulin, blood glucose and plasma lipids in *db/db* mice. Daily rhythms of corticosterone and insulin were monitored during a 24-h period in lean and *db/db* mice following 14 daily injections of BC/SKF or vehicle at 1 HALO (fig. 4). We found the following: (i) In the *db/db* mouse, the mean 24-hour corticosterone level was significantly increased with a broadened daily peak relative to lean controls. (ii) In treated *db/db* mice, the overall 24 h mean value of corticosterone was reduced (23%, $P < 0.05$), and the acrophase (daily peak) was shifted towards normal. (iii) BC/SKF treatment significantly enhanced the plasma

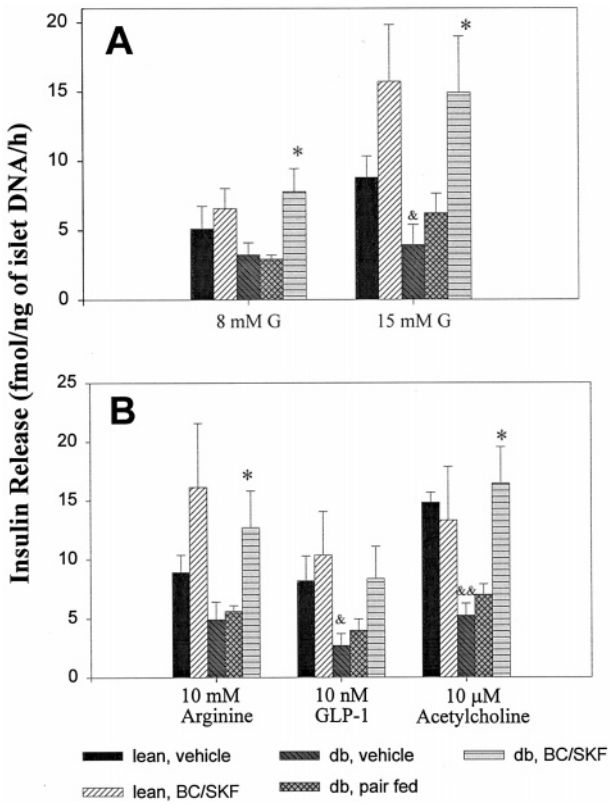

Figure 2. Insulin release from pancreatic islets in static incubation. (*A*) Glucose-stimulated insulin release, (*B*) nonglucose secretagogue-induced insulin release from islets incubated in the medium containing 8 mM glucose. Considering the difference in the size of the islets isolated from lean and *db/db* mice, insulin release data were standardized by islet DNA content. $^{\&}P < 0.05$, compared with normal lean mice. $^{\&\&}P < 0.01$, compared with normal lean mice. $^{*}P < 0.05$ compared with both the *db/db* controls and pair fed groups (ANOVA, $n = 6$).

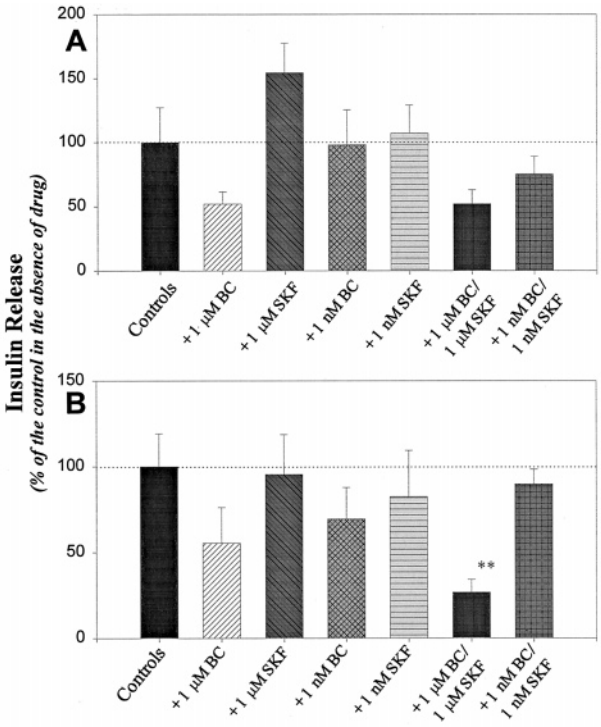

Figure 3. The direct effect of BC/SKF on insulin release from pancreatic islets isolated from untreated *db*/*db* mouse. (*A*) Insulin release from islets incubated with 8 mM glucose. (*B*) Insulin release from islets incubated with 15 mM glucose. Insulin release from islets in the absence of drugs was 1.69 and 2.81 ng/islet/h at 8 and 15 mM glucose, respectively. $^{**}P < 0.01$ compared with that in the controls at the same glucose concentration ($n = 7$).

insulin level at all the time points measured ($P < 0.01$) except 0 HALO. The 24-h profiles of blood glucose and plasma lipids of experimental animals were also examined, as shown in figure 4. In the BC/SKF-treated *db*/*db* mice, the glucose, FFA and triglyceride levels were most markedly reduced (45–60%) during the light period of the 24-h cycle ($P < 0.01$).

Discussion

This study is the first to demonstrate that dopaminergic agonists improve abnormally reduced islet insulin responsiveness to glucose and secretagogues in *db*/*db* mice to levels comparable with those found in islets from lean mice. This amelioration of islet function was associated with fourfold higher insulin content in pancreatic tissue and decreases in blood glucose and plasma lipids, compared with untreated *db*/*db* mice.

As stated, the *db*/*db* mouse is an obese diabetic animal model characterized by islet dysfunction. Exhaustion of pancreatic β cells gradually results in development of lethal diabetes. The reason for the failure of pancreatic islets in the *db*/*db* mouse is obscure. There is a marked reduction in the GLUT2 expression in these islets [17]. However, it has been reported that when islets from *db*/*db* mice were transplanted to normal mice, GLUT2 expression in these islets was partly recovered, indicating that a diabetic environment might have an impact on islet dysfunction [17]. This diabetic environment of *db*/*db* mice includes hyperglycaemia and hyperlipidaemia, which may be the result of an altered hormone balance and abnormalities in the central nervous system [18]. For example, (i) glucose utilization in the hypothalamus of the *db*/*db* mouse is significantly impaired by 4 weeks of age [19]; (ii) there is a higher level of degenerated neurons in the arcuate nuclei and VMH [20]; (iii) the norepinephrine level in the VMH is markedly increased in the *db*/*db* mouse [21, 22]; (iv) there is a dramatic increase in the serum corticosterone level that may potentiate insulin resistance and obesity [23, 24]; and (v) the *db*/*db* gene represents a functional mutation of the leptin receptor which has been associated with hypothalamic abnormalities regulating metabolism [25, 26]. Collectively, these activities drive increases in serum glucose and FFA [9], which may induce glucose and lipid β-cell toxicity, respectively [1–4].

Accordingly, abnormalities in the neuroendocrine system may have a substantial impact on islet dysfunction by producing changes in the metabolic milieu and/or by directly influencing insulin release. Previous studies have shown that dopaminergic agonists influence central circadian neuroendocrine activities regulating metabolism to reduce insulin resistance [6, 27]. This dopaminergic influence is associated with (i) a marked decrease in the circadian peak of hepatic glucose output and lipolysis associated with a decrease in VMH noradrenergic stimulation of these activities [6, 9, 28], and (ii) a strong reduction in hypothalamic neuropeptide Y (NPY) messenger RNA (mRNA) and protein [29, 30], two activities known to potentiate hyperglycaemia and hyperlipidaemia, as well as insulin resistance and diabetes. Moreover, timed dopaminergic stimulation normalizes the circadian rhythm of corticosterone release in obese, insulin-resistant animals [31]. Corticosterone functions to modulate central circadian oscillations [32] and to directly influence lipid synthesis and insulin sensitivity. Reduction of high levels of corticosterone in *ob*/*ob* mice by adrenalectomy results in substantial reductions of obesity and insulin resistance [33], possibly attributed to concurrent reduction in hypothalamic NPY levels [34]. In the present study as well, the administration of dopamine D_2/D_1 receptor agonists adjusted the phase of daily cortico-sterone rhythm towards that of lean mice and reduced the elevated level of serum corticosterone. BC/SKF treatment also markedly reduced the diurnal (light period) levels of glucose, FFA

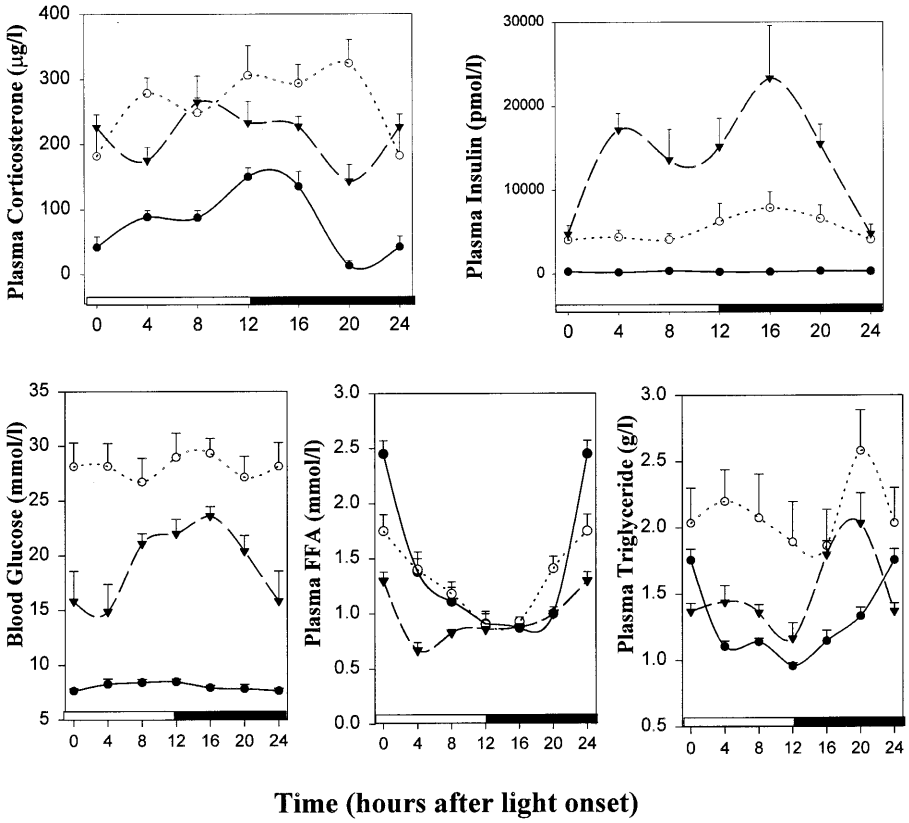

Figure 4. The daily rhythms of corticosterone, insulin, blood glucose and plasma lipids in plasma of *db*/*db* mice and their lean littermates. The plasma concentrations of corticosterone, insulin, blood glucose, plasma FFA and plasma triglyceride for a 24-h period were measured in lean (●——●), vehicle-injected *db*/*db* (○·····○) and BC/SKF-treated *db*/*db* (▼ – – – ▼) mice. Each data point represents the mean of six values. Two-way ANOVA showed a significant ($P < 0.01$) difference among lean, vehicle-injected and BC/SKF-treated *db*/*db* mice in the daily rhythms of all parameters.

and triglyceride (fig. 4), an effect consistent with reducing the noradrenergic VMH drive for these activities, which peak at this time of day [35]. Thus, BC/SKF treatment reduces elevated VMH noradrenergic activity and alters other hypothalamic centres to normalize the neuroendocrine axis regulating metabolism. Such activities would and do concurrently reduce hyperglycaemia and hyperlipidaemia as described above (see table 1 and fig. 4) and consequently remove glucose and lipid toxicity effects on pancreatic β cells. Furthermore, a role for neuroendocrine involvement in islet response to BC/SKF treatment resides in the fact that insulin secretion in the presence of acetylcholine was markedly enhanced (to levels seen in lean mice), suggesting improvement of autonomic parasympathetic regulation of insulin secretion.

We examined the effect of reducing hyperglycaemia independent of hyperlipidaemia on islet insulin release. In pair-fed mice, the food restriction reduced blood glucose to a level similar to that in BC/SKF-treated mice but did not reduce plasma FFA levels. In these pair-fed mice neither the plasma insulin level nor the secretagogue-induced insulin release in vitro was increased compared with that in untreated mice. Thus, a decrease in blood glucose alone does not improve islet function. Pair feeding actually increased plasma FFA levels by 25%, which may explain the lack of a secretagogue-induced insulin response in islets isolated from these mice, since hyperlipidaemia can induce a blunted glucose-stimulated insulin release from pancreatic islets [36–40]. Therefore, concurrent reductions of both glucose and lipid levels in the blood may be a key mechanism by which BC/SKF treatment improves islet function in *db*/*db* mice.

Our present study provides two arguments supporting the postulate that the mechanism of BC/SKF action on islet function occurs through improving the diabetic environment and/or by adjusting abnormal central mechanisms regulating metabolism, rather than directly affecting pancreatic islets. First, BC/SKF treatment showed profound effects in *db*/*db* mice but not in their lean littermates, which exhibit a normal central nervous

system and energy metabolic profile. Second, when added directly to incubated islets, BC/SKF did not have a direct stimulatory effect on insulin release from incubated islets. BC/SKF at 1 μM concentration actually inhibited insulin release, which is consistent with reports from other laboratories [41–43]. The mechanism of dopamine agonist-induced inhibition of insulin release is not yet clear, but it may be related to Ca^{2+} efflux from pancreatic β cells [44, 45]. A similar phenomenon has also been observed in vivo only shortly after acute injection of dopamine [46]. Islets used in this study were collected 24 h after the last BC/SKF injection; thus the likelihood of a direct effect is unsupported.

In *db*/*db* mice, exhaustion of pancreatic islets (loss of insulin response to glucose) results in relative insufficiency of insulin under severe hyperglycaemic conditions. This situation has been improved by BC/SKF treatment, as shown by a 3.6-fold higher insulin level in treated mice compared with that in a vehicle group, although blood glucose was reduced from 33.6 to 19.2 mM in treated mice. This in vivo finding is consistent with our observations of improved secretagogue-stimulated insulin release from isolated islets of BC/SKF-treated mice.

In summary, administration of BC/SKF ameliorated the hyperglycaemic and hyperlipidaemic condition in *db*/*db* mice and markedly improved islet insulin release capacity in vitro to a level observed in normal mice. This improvement is possibly due to an effect on regulatory mechanisms in the hypothalamic-neuroendocrine system, which in turn reduce glucose and lipid β cell toxicity.

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