NN414, a SUR1/Kir6.2-Selective Potassium Channel Opener, Reduces Blood Glucose and Improves Glucose Tolerance in the VDF Zucker Rat

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A novel potassium channel opener compound, NN414, selective for the SUR1/Kir6.2 subtype of the ATP-sensitive potassium channel, was used to examine the effect of reducing β-cell workload in the male Vancouver diabetic fatty (VDF) Zucker rat model of mild type 2 diabetes. Two chronic dosing protocols of NN414 of 3 weeks' duration were compared with appropriate vehicle-treated controls. In the first group, rats received NN414 (continued group; 1.5 mg/kg p.o. twice daily), during which an oral glucose tolerance test (OGTT) (on day 19 of dosing) was performed and insulin secretion from an in situ perfused pancreas preparation (on day 21) was measured. The second group received NN414 (discontinued group; same dose), but active treatment was replaced by vehicle treatment 2 days before the OGTT and for a further 2 days before the perfused pancreas study. Basal glucose was significantly reduced by NN414, with the fall averaging 0.64 mmol/l after 3 weeks of treatment ($P < 0.0001$). The glucose excursion and hyperinsulinemia during the OGTT were significantly different between the continued, discontinued, and vehicle groups (glucose area under the curve [AUC]: 640 ± 29, 740 ± 27, and 954 ± 82 mmol · l^{-1} · min^{-1}, respectively, $P < 0.0001$; insulin AUC: 38.9 ± 4.2, 44.2 ± 4.2, and 55.1 ± 2.6 nmol · l^{-1} · min^{-1}, respectively, $P < 0.0001$). Hyperinsulinemia during the pancreas perfusion with 4.4 mmol/l glucose was significantly reduced in both treatment groups versus vehicle ($P < 0.0005$). Insulin secretory responsiveness to a step increase in glucose from 4.4 to 16.6 mmol/l, calculated relative to basal, was significantly improved in the continued group versus vehicle ($P < 0.01$). In conclusion, administration of NN414 for 3 weeks in VDF rats reduces basal hyperglycemia, improves glucose tolerance, and reduces hyperinsulinemia during an OGTT and improves insulin secretory responsiveness ex vivo. NN414 may therefore represent a novel approach to the prevention and treatment of impaired glucose tolerance and type 2 diabetes.
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AUC, area under the curve; CISI, composite insulin sensitivity index; K_{ATP}CO, ATP-sensitive potassium channel opener; OGTT, oral glucose tolerance test; PIR, peripheral insulin resistance.

The pancreatic defect in type 2 diabetes is characterized by a loss of the first phase of insulin secretion in response to a secretory stimulus (1–3) and is probably the most noticeable feature of pancreatic secretory dysfunction, which also encompasses impaired pulsatile insulin release (4). Abnormal first-phase insulin secretion leads to an inappropriately low suppression of hepatic glucose production and, therefore, to an enhanced level of systemic glycemia, which consequentially causes further demand on the β-cell, producing hyperinsulinemia in the longer term. The role of hyperinsulinemia in the pathogenesis of the disease is controversial, being either a consequence of elevated peripheral insulin resistance (PIR) (5) or a primary cause, resulting in downregulation of peripheral sensitivity to insulin, or both. In both cases, gradually worsening PIR acts in concert with primary pancreatic events to feed back positively on the workload of the pancreatic β-cell, thus further exacerbating hyperinsulinemia in a vicious circle of events that culminates, eventually, in β-cell exhaustion and onset of frank diabetes (4). Agents that reduce β-cell workload and thereby reduce hyperinsulinemia may therefore be therapeutically useful, by reducing the impact of this vicious circle on the development and exacerbation of type 2 diabetes.

It has been known for >20 years that ATP-sensitive potassium channel openers (K_{ATP}COs) can improve the insulin release pattern in human type 2 diabetes. This effect is thought to be mediated via hyperpolarization of β-cells, thereby providing β-cell rest by reducing insulin release. Thus, diazoxide has been shown to improve the release of insulin in response to intravenously administered glucagon and tolbutamide in type 2 diabetic patients (6). This early observation has more recently been confirmed by Guldstrand et al. (7), who demonstrated an improvement in first-phase insulin release in response to an arginine challenge in type 2 diabetic patients treated with diazoxide. Furthermore, improvement in the type 2 diabetes–induced insulin secretory defect has been observed in response to a hyperglycemic clamp after acute β-cell rest induced by a somatostatin infusion (8). Moreover, there is evidence to support the contention that β-cell rest may actually prevent the onset of diabetes. Thus, preclinically, it is known that diazoxide protects 90% pancreatectomized rats from impaired glucose-stimulated insulin release and increases pancreatic insulin content by

50% (9). Furthermore, diazoxide prevents sucrose-induced diabetes in rats (10). Together, these results suggest that inhibition of insulin release by use of K_{ATP}CO compounds will ameliorate insulin secretory capacity. However, it is still debatable for how long β-cell rest has to be maintained to see improvements in insulin secretory capacity.

It remains uncertain whether the effects of diazoxide are mediated via the β-cell or whether some other effect of the compound is responsible, because diazoxide is equally active on potassium channels incorporating the SUR1, SUR2A, and SUR2B subunits that are found in pancreatic, cardiac, and vascular tissues, respectively. Therefore, we used a novel orally active compound, selective for the SUR1/Kir6.2 potassium channel (11), NN414 [3-(1-methylcyclopropyl)amino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide], which is related pharmacologically to similar probe compounds, e.g., NNC 55-0118 (12,13), to study the specific effects of SUR1/Kir6.2 activation on the β-cell in vivo.

RESEARCH DESIGN AND METHODS

Animals and drug administration regimen. The VDF Zucker (*fa/fa*) rat strain has been established at the Department of Physiology, University of British Colombia (14). Animals are characterized by hyperinsulinemia and marked obesity but, unlike conventional Zucker obese rats, have mild diabetes. Age-matched (10–12 weeks old at the start of the dosing period) male obese animals (400–475 g) were used. Rats were initially allocated to groups of eight animals in each of four experimental groups (two treatment groups and two control groups), housed in standard 12-h light/12-h dark conditions, and given free access to food and water ad libitum.

Protocol. NN414 was dissolved in vehicle (10% glycerol, 0.5% gelatin, and 10% cellulose in water) to give a stock solution of 1 mg/ml, which was prepared freshly every week. NN414 was administered orally in a dose volume of 1 ml/kg according to the following three dosing regimens:

Continuous dosing protocol. Rats received NN414 at a dose of 1.5 mg/kg twice daily (8:00 A.M. and 5:00 P.M.) each day for 3 weeks. The dosing was continued until the 5:00 P.M. dose on day 18, and the oral glucose tolerance test (OGTT) was performed at 8:00 A.M. on day 19. Dosing was then resumed at 8:00 A.M. and 5:00 P.M. until day 20; the animals were killed before the in situ perfused pancreas study on the morning of day 21.

Discontinuous dosing protocol. Rats received NN414 at a dose of 1.5 mg/kg twice daily (8:00 A.M. and 5:00 P.M.) each day for 3 weeks. The dosing was discontinued for 2 days before the OGTT, followed by a further 2 days before the day animals were killed for the isolated perfused pancreas study. Rats were dosed with vehicle only during the period of drug discontinuation.

Two vehicle-treated groups. Rats received vehicle (1 ml/kg b.i.d.) at each time point corresponding to each of the treatment groups above. Data from these two groups were merged for the purposes of data analysis (control group).

Within each group, rats were dosed using a staggered daily dosing regimen that allowed each OGTT and isolated perfused pancreas study to be performed between 8:00 and 10:00 A.M.

Blood glucose, blood insulin, and body weight measurement. Blood glucose was measured 5 min before each dose of NN414. Blood samples (2 × 50 μl) were obtained for insulin measurement by sampling from the tip of the tail. The insulin radioimmunoassay and its characterization have been described elsewhere (15). Body weight was measured daily 10 min before each dose.

OGTT. Rats were fasted overnight, and on the morning of the study, samples were taken for determination of fasting blood glucose and insulin. An oral glucose challenge (1 g/kg) was administered to conscious unrestrained animals, and blood glucose was measured at 10, 20, 30, 60, and 120 min after glucose ingestion. Blood glucose was measured using a Surestep monitoring system (LifeScan, Burnaby, BC, Canada).

A composite insulin sensitivity index (CISI) was estimated from the OGTT, adapting the approach proposed by Matsuda and DeFronzo (16) and previously applied to VDF rats (17). CISI was calculated using the following equation:

$$\mathrm{CISI} = 10{,}000/(\mathrm{FBG} \times \mathrm{FPI} \times \mathrm{MG} \times \mathrm{MI})^{1/2}$$

where FBG is fasting blood glucose, FPI is fasting plasma insulin, and MG and MI represent the mean glucose and mean insulin concentrations during the course of the OGTT (area under the curve [AUC]/60 min). The factor 10,000 represents an arbitrary scaling constant.

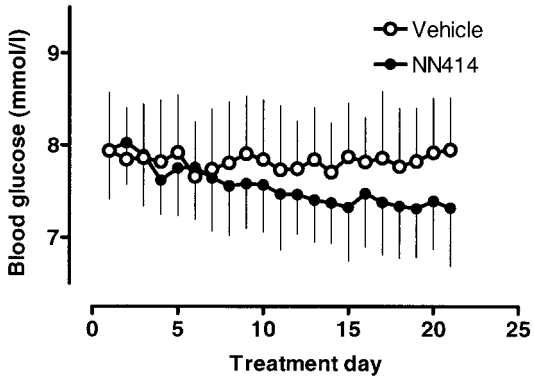

FIG. 1. Blood glucose levels obtained during treatment with either NN414 (1.5 mg · kg^{-1} · day^{-1} b.i.d. for 3 weeks; ●) or vehicle in VDF rats (○). Data from both the continuous and discontinuous dosing regimens were combined and shown as the treated group. Data are means ± SD.

Isolated perfused pancreas. Animals were fasted overnight with the last dose of NN414 administered at 5:00 P.M. the previous evening. Pancreas isolation is described in detail elsewhere (15). Briefly, rats were anesthetized (60 mg/kg pentobarbital intraperitoneally) and the pancreas and associated duodenum were isolated. The perfusate was a modified Krebs-Ringer buffer containing 3% dextran (Sigma) and 0.2% radioimmunoassay grade BSA gassed with 95% oxygen and 5% carbon dioxide at pH 7.4, and supplied at a flow rate of 4 ml/min. First, a 10-min equilibration period with glucose concentration at 4.4 mmol/l was established, following which samples of emergent perfusate were collected at 1-min intervals for 6 min. The glucose concentration in the perfusate was then stepped up to 16.6 mmol/l for the next 34 min, and samples were collected every 30 s for the first 10 min of high glucose (for a more complete analysis of the first-phase insulin secretion) and every 1 min for the remainder of the perfusion period.

Statistical analysis. Statistic analyses were performed with the SAS system for Windows, release 8.00. AUC was calculated using the trapezoidal rule. Means and SD were calculated. Statistical analysis of CISI was performed on reciprocal data (1/CISI) to obtain equal variances between groups. Student's unpaired *t* test, paired *t* test, or ANOVA with Tukey's studentized range test for post hoc pairwise comparisons were used as indicated. $P < 0.05$ was taken to indicate statistical significance.

RESULTS

Effect of NN414 on blood glucose levels throughout the dosing period. Blood glucose levels were similar in all four groups before initiation of treatment (NS by ANOVA). For the first 3 weeks, treatment was identical in the continued and discontinued NN414 groups, and, therefore, the analysis for parameters measured on day 21 was performed with the two treated groups merged and compared with the two vehicle groups combined. In the vehicle-treated animals, blood glucose was stable with no difference between day 1 and 21 (0.01 ± 0.22 mmol/l, $P = 0.83$, paired *t* test). Conversely, in the 16 NN414-treated animals, glucose decreased progressively, with the difference between blood glucose on day 1 and 21 being significant (−0.63 ± 0.48 mmol/l, $P < 0.0002$, paired *t* test). Compared with the control group, NN414 significantly reduced blood glucose, with the average reduction amounting to 0.64 mmol/l by day 21 ($P < 0.0001$, unpaired *t* test). The basal blood glucose data are illustrated in Fig. 1.

Effect of NN414 on body weight throughout the dosing period. No significant differences in body weight were observed between vehicle- and NN414-treated animals. The data are shown in Fig. 2.

Effect of NN414 on fasting blood glucose and OGTT. Immediately before administration of oral glucose, at

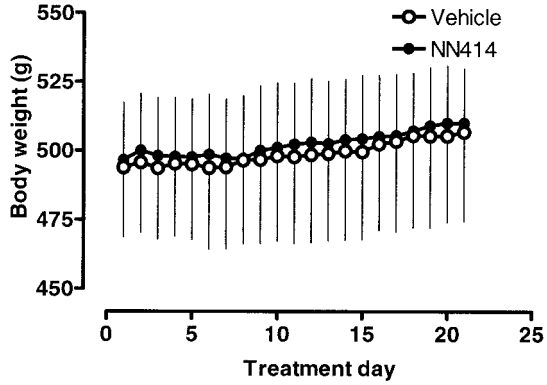

FIG. 2. Body weight measured during treatment with either NN414 (1.5 mg · kg^{-1} · day^{-1} b.i.d. for 3 weeks ●) or vehicle in VDF rats (○). Data from both the continuous and discontinuous dosing regimens were combined and shown as the treated group. Data are means ± SD.

which time treatment with NN414 had been withheld for 2 days in the discontinued group, fasting blood glucose levels differed significantly between all three groups (6.5 ± 0.6, 7.8 ± 0.6, and 8.7 ± 0.7 mmol/l in the continued and discontinued treatment groups and the controls, respectively; $P < 0.0001$ by ANOVA, $P < 0.0001$ continued vs. control, $P < 0.05$ discontinued vs. control, $P < 0.0005$ continued vs. discontinued). Both NN414 treatment groups had a lower AUC of blood glucose levels during OGTT compared with controls. Furthermore, the AUC for the continued group was lower than the AUC for the discontinued group (640 ± 29, 740 ± 27, and 954 ± 82 mmol · l^{-1} · min^{-1} in the continued and discontinued treatment groups and the controls, respectively; $P <$ 0.0001 by ANOVA, $P < 0.001$ continued and discontinued vs. control, $P < 0.01$ continued vs. discontinued). The data are illustrated in Fig. 3*A*.

Effect of NN414 on fasting and OGTT insulin levels. Fasting insulin levels also differed before the OGTT (464 ± 87, 543 ± 76, and 767 ± 49 pmol/l in the continued and discontinued treatment groups and the controls, respectively; $P < 0.0001$ by ANOVA, $P < 0.0001$ continued and discontinued vs. control, $P < 0.05$ continued vs. discontinued). The insulin excursion for both NN414 treatment groups was reduced compared with controls, with the continued group having a smaller AUC than the discontinued group (insulin AUC, 38.9 ± 4.2, 44.2 ± 4.2, and 55.1 ± 2.6 nmol · l^{-1} · min^{-1} in the continued and discontinued treatment groups and the controls, respectively; $P <$ 0.0001 by ANOVA, $P < 0.001$ continued and discontinued vs. controls, $P < 0.01$ continued vs. discontinued). The data are illustrated in Fig. 3*B*.

Effect of NN414 on CISI. Insulin sensitivity judged by the CISI was significantly improved in both treated groups (continued, 2.23 ± 0.27; discontinued, 1.63 ± 0.18; control, 1.02 ± 0.06 [arbitrary units]; $P < 0.0001$ by ANOVA).

Effect of NN414 on the pattern of insulin release from the isolated perfused pancreas preparation. Treatment with NN414 significantly lowered the basal level of insulin release from the isolated perfused pancreas. Thus, the AUCs for perfusate insulin concentration were 20.9 ± 3.6, 24.2 ± 7.2, and 54.1 ± 26.5 nmol · l^{-1} · min^{-1} in the continued and discontinued treatment groups and the controls, with differences being statistically significant ($P < 0.0005$ by ANOVA, $P < 0.005$ continued vs.

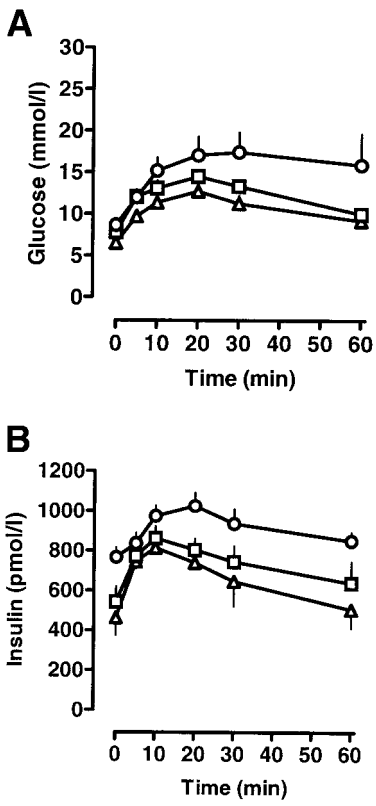

FIG. 3. Ameliorative effect of NN414 (1.5 mg · kg^{-1} · day^{-1} b.i.d. for 3 weeks) on oral glucose tolerance (*A*) and insulin levels during OGTT (*B*) after both continuous and discontinuous dosing regimens. Data from vehicle-treated rats (○), discontinued NN414-treated rats (□), and continued NN414-treated rats (△) are shown. Data are mean ± SD.

controls, $P < 0.01$ discontinued vs. controls). The glucose-dependent first-phase insulin release was assessed by expressing each data set as percent of basal, defined as the mean insulin concentration from each pancreas during the first 6-min perfusion period after the equilibration period. Thereafter, the AUC from 6 to 16 min (the predefined period during which insulin was measured every 30 s to assess first-phase release) was calculated. This normalized AUC is a measure of the insulin secretory responsiveness to the given glucose concentration change. First-phase insulin secretion defined in this way was 2,161 ± 361, 1,684 ± 431, and 1,435 ± 554%×min in the continued and discontinued treatment groups and the controls, respectively ($P < 0.01$ by ANOVA, $P < 0.01$ continued vs. controls). Perfused pancreas data are shown in Fig. 4.

DISCUSSION

Diazoxide has been known for many years to improve the pattern of insulin release, but its potential as a therapeutic agent for treatment of type 2 diabetes has been limited because of its interaction with multiple potassium channel subtypes and consequential side effects (e.g., edema). Attempts to make fully SUR1/Kir6.2-selective compounds have been made previously (18,19), culminating in the discovery of NN414, which is both selective for SUR1/Kir6.2 and orally active (11). NN414, therefore, was selected for use as a probe in these investigations.

The present study demonstrates that chronic treatment with NN414 results in a progressive antidiabetic effect and

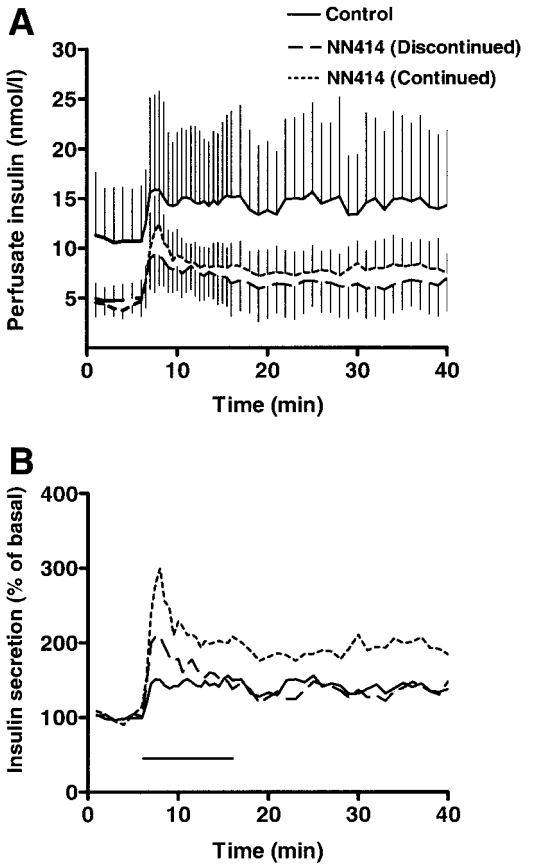

FIG. 4. Potentiation of first-phase insulin secretion from isolated perfused pancreas from either vehicle- or NN414-pretreated rats (1.5 mg · kg^{-1} · day^{-1} b.i.d. for 3 weeks) after both continuous and discontinuous dosing regimens. Data are means ± SD. *A*: Absolute insulin concentrations measured in perfusate fractions. *B*: Insulin secretion expressed relative to basal level during the first 6 min with the bar indicating the 30-s sampling period used to estimate first-phase insulin secretion. Glucose concentration in the perfusate was raised from 4.4 to 16.6 mmol/l after 6 min. SD bars have been omitted in *B* for clarity. The solid line shows the data from vehicle-treated rats, the long dashed line shows data from the discontinued NN414-treated rats, and the short-dashed line shows data from the continued NN414-treated rats.

improves both the first-phase insulin release ex vivo and glucose tolerance in the mildly diabetic VDF rat strain. K_{ATP}CO-induced β-cell rest may therefore have an effect on restoring the capacity of the β-cell to produce an adequate first-phase insulin release in response to glucose.

The use of NN414 as a pharmacological probe for inducing β-cell rest, commensurate with protracted use in vivo, is supported by a preliminary report showing tolerability and selectivity for suppression of insulin secretion in type 2 diabetic patients (20). It is probable that NN414 has direct effects on the β-cell because the compound has been designed to be active selectively at the SUR1/Kir6.2 subtype of the potassium channels that are present on pancreatic β-cells (11). However, we should bear in mind that SUR1 subunits are found at additional sites, meaning that SUR1/Kir6.2 selectivity does not necessarily confer β-cell selectivity. For example, SUR1/Kir6.2 channels are also found in α-cells, and, in unpublished studies, we have observed that a dose-dependent suppression of glucagon release by NN414 can be observed in isolated rat pancreas. The beneficial effects of NN414 reported here may therefore have been due to its interaction with pancreatic cells of different types and, indeed, nonpancreatic SUR1/Kir6.2 channels.

Most studies (9,21,22) looking at the beneficial effects of diazoxide in vivo have been conducted 2 or more days after cessation of an extended dosing period (equivalent to the discontinuous protocol in the present study). However, it remains unknown whether K_{ATP}CO therapy also has beneficial effects during the actual dosing period. For this reason, we included a group where the responses to the K_{ATP}CO were studied during the course of the dosing cycle (continuous dosing protocol) to investigate whether benefits of K_{ATP}CO therapy could also be seen under these conditions. Proof that benefits of pharmacologically induced β-cell rest exist during the dosing period, in addition to the period after cessation of dosing, is important when considering the clinical potential of K_{ATP}CO therapy.

The observation that fasting blood glucose and plasma insulin are lower in both NN414-treated rats and control-treated rats suggests an improvement in glucose tolerance—an observation confirmed by an improvement in OGTT. This ameliorative effect of K_{ATP}CO drugs on glucose tolerance has also been demonstrated with the nonselective K_{ATP}CO, diazoxide, when administered chronically. Diazoxide (100 mg · kg^{-1} · day^{-1} for 8 weeks) reduced postabsorptive insulin levels and body weight gain in hyperinsulinemic obese Zucker rats compared with pair-fed controls, despite the fact that food intake increased slightly (22). Glucose tolerance was improved and postabsorptive glucose concentrations were lowered by the chronic diazoxide treatment. Leahy et al. (9) reported that glucose tolerance was improved in 90% pancreatectomized rats treated with diazoxide (30 mg/kg p.o. twice daily for 5 days), whereas Aizawa et al. (21) have shown that chronic administration of diazoxide (0.2% in diet) for 8 weeks to prediabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats prevents the development of insulin resistance and obesity in this model. In all of these studies, diazoxide treatment was terminated for 2 days before measurement of the beneficial effects of the compound.

A further aspect of the control of PIR by the pancreas is the question of whether an improvement in the insulin release pattern per se might be part of the explanation for the long-term effect of the compound on insulin sensitivity. We therefore studied the effects of the K_{ATP}CO on first-phase insulin release to investigate this possibility. An improved β-cell responsiveness to glucose was observed in the perfused pancreas relative to baseline secretion. In absolute terms, however, no difference in pancreatic insulin output was observed between the groups during the step-up in glucose concentration, despite an apparent improvement in the continuously dosed group. Our interpretation that an improvement in first-phase insulin release exists as a result of NN414 treatment relies on the enhancement of insulin release relative to the basal release. However, in view of the hyperbolic relationship that exists between insulin secretion and insulin action (23), it is reasonable to interpret the difference in percent change as an improved β-cell responsiveness viewed in relation to the prevailing degree of insulin resistance. This observation is in agreement with the hypothesis that the effect of

K_{ATP}CO treatment on OGTT and first-phase insulin release contributes to the antidiabetic effect seen in these studies with NN414 and in the published studies with diazoxide. The relationship between β-cell rest and resultant improvements in insulin secretory patterns has been studied in the clinic by use of simultaneous somatostatin infusion and hyperglycemic clamp and by observation of the effect of termination of the somatostatin infusion on resultant induced adverse insulin secretory patterns in type 2 diabetic patients (8). Somatostatin pretreatment improved the insulin secretory pattern compared with placebo-infused control subjects. We therefore speculate that the effect of the K_{ATP}CO drug might mimic the effects of somatostatin by causing β-cell rest and consequential improvement in pancreatic function and that the amelioration of adverse glucose tolerance is secondary to long-term improvements in β-cell function.

The progressive antidiabetic effect of NN414 on the mildly diabetic state of the VDF rat is suggestive that the long-term benefit of K_{ATP}CO drugs on the β-cell is mediated via the SUR1/Kir6.2 channel, because the compound has been designed to act selectively at this subtype, and diazoxide, used in previous studies, does not act selectively at this subtype of channel.

Recent evidence is emerging that a discrete genetically determined increase in the open probability of SUR1/Kir6.2 may predispose affected people to develop type 2 diabetes (24,25) via a mechanism that reduces insulin release (26) and glucose-induced glucagon suppression (27). These observations suggest that constitutive activation of Kir6.2 could, at least in theory, predispose an individual to type 2 diabetes—an observation that might be viewed as somewhat contradictory with the present suggestion for a therapeutic application of K_{ATP}CO-opening agents. Our findings that a periodical opening of potassium channels improves β-cell function during a meal do not necessarily contradict these observations in patients with permanently affected channels, suggesting that some periodicity with respect to potassium channel opening is an important determinant of the consequences on pancreatic function. Appropriate clinical studies, or studies involving genetically manipulated animals, may shed further light on this issue.

In conclusion, an antidiabetic effect of NN414 has been demonstrated both during and after cessation of K_{ATP}CO treatment in a strain of mildly diabetic rats. It is possible to speculate that β-cell rest, induced pharmacologically, might interrupt a vicious circle of events whereby pancreatic overactivity culminates in progression and exacerbation of type 2 diabetes. We propose, therefore, that SUR1/Kir6.2-selective K_{ATP}CO drugs such as NN414 may have utility in the treatment (and prevention) of human type 2 diabetes.

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