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Effects of Aminoguanidine on Rat Pancreatic Islets in Culture and on the Pancreatic Islet Blood Flow of Anaesthetized Rats

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ABSTRACT. Aminoguanidine (AG; ≤0.5 mM) is a potent inhibitor of the inducible form of nitric oxide synthase (iNOS) and, at higher concentrations, is also able to prevent advanced glycosylation of proteins. Due to these properties, AG might be an interesting therapeutic compound for prevention of the development of diabetes and for prevention of diabetes complications. In the present study, we examined the effect of AG (0.1, 0.5, 1.0, 5.0, or 10 mM) on prolonged *in vitro* culture of isolated rat pancreatic islets. Furthermore, the acute effect of AG on pancreatic and islet blood flow in anaesthetized rats was studied with a microsphere technique. Culture for 6 days of pancreatic islets at either 11.1 mM or 28 mM glucose, in the presence of 0.1–1.0 mM AG, was not toxic to the islet cells or impaired insulin secretion. However, when islets were cultured for 8 days with the addition of 5 mM AG at 11.1 mM or 28 mM glucose, a 50% inhibition of glucose-stimulated insulin release was observed. Rats injected intravenously with AG (1, 10, or 50 mg/kg body weight) had a decreased pancreatic blood flow 30 min later. Glucose injection (1 g/kg body weight) increased the islet blood flow, and this effect was not attenuated by AG. The present data suggest that AG, when used in concentrations that inhibit iNOS, can affect pancreatic blood flow, but appears not to be directly harmful to β-cell function. BIOCHEM PHARMACOL 51;12:1711–1717, 1996.

KEY WORDS. aminoguanidine; blood flow; insulin release, nitric oxide; pancreatic islets

It has been proposed that NO† is involved in the mechanisms causing pancreatic β-cell damage during the development of IDDM [1, 2]. One tentative strategy to prevent IDDM could, therefore, be to block the NO-generating enzyme NOS, which exists in a constitutive cNOS and in an inducible iNOS form [3, 4]. Indeed, it has been reported that two NOS inhibitors (L-N^G-monomethyl-arginine and L-NAME) prevented diabetes induced by low-dose injections of streptozotocin in mice [5, 6]. AG is a potent inhibitor of the inducible form of NOS in particular [7–9]. Moreover, it was recently reported that AG attenuated endotoxin-induced uveitis in rats [10] and ameliorated experimental autoimmune encephalomyelitis in mice [11], thereby suggesting a role for iNOS in the development of autoimmune disease. In addition, it is well known that AG can diminish advanced glycosylation of proteins, which might be beneficial in preventing chronic diabetic complications [12, 13]. However, when AG was administered to IDDM-prone nonobese diabetic mice [14] or BioBreeding rats [15], or in conjunction with low-dose streptozotocin injections in C57BL/Ks mice [16], it failed to decrease the incidence of IDDM. In the latter experimental model, the diabetic state was even aggravated by AG [16]. The reason for this effect is unknown, but it could be that AG itself influences β-cell function. Another possibility is that AG affects the pancreatic islet blood circulation, which could influence the infiltration of immune cells in the pancreatic islets in IDDM and/or the local islet concentrations of soluble inflammatory mediators contributing to β-cell destruction. To study these issues further, we have examined the direct effect of prolonged exposure to AG on the function of pancreatic islets in culture, and of AG administration *in vivo* on pancreatic and islet blood flow in rats. In some *in vitro* experiments, the effect of AG was examined at two different glucose concentrations (11.1 mM and 28 mM) because previous data suggest that glucose can modify a possible stressful action on islet β-cells *in vitro* [17–19].

MATERIALS AND METHODS

Chemicals

Chemical purchased from Boehringer-Mannheim (Mannheim, Germany) was collagenase prepared from *Clostridium histolyticum*. From HyClone (Cramlington, U.K.), culture

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† *Abbreviations:* AG, aminoguanidine; cNOS, constitutive nitric oxide synthase; FCS, fetal calf serum; iNOS, inducible nitric oxide synthase; IDDM, insulin-dependent diabetes mellitus; KRBH, Krebs-Ringer bicarbonate buffer supplemented with 2 mg/mL BSA and 10 mM HEPES; L-NAME, N-nitro-L-arginine-methylester; NO, nitric oxide; NOS, nitric oxide synthase; RIA, radioimmunoassay.

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medium RPMI 1640 (11.1 mM glucose) and FCS from Miles (Slough, U.K.), BSA; from Amersham International (Amersham, U.K.), L-[4.5-^{3}H]leucine and D-[U-^{14}C]glucose; from Sigma Chemicals (St. Louis, MO, U.S.A.), N^G-nitro-L-arginine, HEPES, and antimycin A; from New England Nuclear (Boston, MA, U.S.A.), hyamine hydroxide; from Novo Industries (Copenhagen, Denmark), a kit for immunoassay for insulin.

Islet Isolation and Culture

Noninbred, adult male Sprague-Dawley rats were used (Biomedical Center, Uppsala, Sweden). Pancreatic islets were isolated by a collagenase digestion procedure as described in detail elsewhere [20]. Groups of 150–200 islets were precultured freefloating for 6–7 days at 37°C in an atmosphere of humidified air + 5% CO_2 in 4.5 mL of medium RPMI 1640 supplemented with 0.5 mL FCS. Medium was changed every second day. Islets in groups of 65–150 were then transferred to new culture dishes containing 4.5 mL RPMI 1640 (11.1 or 28 mM glucose) + 0.5 mL FCS without or with addition of AG at concentrations of 0.1, 0.5, 1.0, 5.0, or 10 mM and cultured for 2, 6, or 8 days.

Islet Medium Insulin Concentration, Islet Glucose-Stimulated Insulin Release, and Islet Contents of Insulin and DNA

Samples of the culture media from cultured islets were collected every second day and kept frozen at −20°C prior to measurement of the medium insulin concentration by radioimmunoassay [21]. After the culture period, triplicate groups of 10 islets were transferred to sealed glass vials containing 0.25 mL KRBH [22]. The islets were incubated at 37°C in a gas phase of 95% O_2 + 5% CO_2 at 1.7 mM glucose for 1 hr. Then, the incubation medium was gently removed and replaced by KRBH supplemented with 16.7 mM glucose, and the incubation continued for another hr. The incubation media were collected and frozen at −20°C before assay. After the incubations, the islets were harvested and pooled in groups of 30 and homogenised in 0.2 mL redistilled water. A fraction of the homogenate was mixed with acid ethanol, and insulin was extracted overnight at 4°C. The insulin concentration of the extract was measured by RIA [21]. Another fraction of the aqueous homogenate was used for DNA measurement [23].

Islet Glucose Oxidation Rate

Groups of 10 islets in triplicate were incubated in 100 μL KRBH without BSA supplemented with D-[U-^{14}C]glucose and nonradioactive glucose, at a concentration of 16.7 mM, in glass vials at 37°C in an atmosphere of 95% O_2 and 5% CO_2 [24]. After 90 min, the incubation was interrupted and 0.05 mM antimycin A added. Liberated $^{14}CO_2$ was trapped in hyamine hydroxide and radioactivity measured by liquid scintillation.

Blood Flow Measurements

This procedure has been described in detail elsewhere [25]. Briefly, male Sprague-Dawley rats (Biomedical Center, Uppsala, Sweden) weighing approximately 350 g were anesthetized with an intraperitoneal injection of pentobarbital sodium (60 mg/kg body weight), heparinized, and placed on an operating table maintained at body temperature (38°C). Polyethylene catheters were inserted into the ascending aorta, *via* the right carotid artery, and into the femoral artery. The former catheter was connected to a pressure transducer (PDCR 75/1; Druck, Groby, U.K.) to allow continuous monitoring of the mean arterial blood pressure. When the blood pressure had remained stable for at least 15 min, an intravenous injection of saline alone or AG dissolved in saline (1, 10, or 50 mg/kg body weight) was given. Thirty minutes later, some of the animals were injected intravenously with D-glucose (1 g/kg body weight). Thirty minutes after the first injection, or 5 min after glucose administration, 1.5–2.0 × 10^5 nonradioactive microspheres (NEN-Trac®; DuPont Pharmaceuticals, Wilmington, DE, U.S.A.), with a diameter of 11 μm, were injected *via* the catheter with its tip in the ascending aorta over a 10-sec period. Starting 5 sec before the microsphere injection and continuing for a total of 60 sec, an arterial blood sample was collected by free flow from the catheter in the femoral artery at a rate of approximately 0.5 mL/min. The exact withdrawal rate was confirmed in each experiment by weighing the sample. The animals were then killed and the pancreas and adrenal glands removed, blotted, weighed, and treated with a freeze-thawing technique [26] that visualizes the pancreatic islets and the microspheres. The microspheres in these organs, including the islets, were then counted. The number of microspheres in the arterial reference sample was determined by transferring the blood to glass microfibre filters (pore size 0.2 μm) and counting the microspheres in a microscope equipped with transmitted light. The blood flow values were calculated according to the formula $Q_{org} = Q_{ref} \times N_{org}/N_{ref}$, where Q_{org} is organ blood flow (mL/min), Q_{ref} the withdrawal rate of reference sample (mL/min), N_{org} is the number of microspheres present in the organ, and N_{ref} is the number of microspheres in the reference sample. Blood flow values based on the microsphere content of the adrenal glands were used to confirm that the microspheres were adequately mixed in the circulation. A difference of <10% in blood flow values between the glands was taken to indicate sufficient mixing.

Statistical Analysis

In the *in vitro* experiments, where islets were incubated in triplicate (glucose-stimulated insulin release and glucose oxidation), a mean was calculated for each experimental group, and this was considered as one separate observation. Furthermore, every islet observation represented different rat islet donors. Values were expressed as means ± SEM, and groups of data were compared using Student's paired

t-test. When multiple comparisons were performed, the data were compared by ANOVA, including Fisher's protected least statistical difference (PLSD) test, using StatView® (Abacus Concepts, Berkeley, CA, U.S.A.).

RESULTS

Culture of Islets for Two Days with AG

In the first series of experiments, pancreatic islets were exposed for 48 hr at 11.1 mM glucose to different concentrations of AG. It was found that lower concentrations of AG (0.1–1 mM) stimulated medium insulin accumulation, while 5 and 10 mM AG did not cause any changes in the medium insulin (Fig. 1). After culture, the glucose-stimulated insulin release of the islets was examined. The control islets increased their insulin secretion about 6-fold at 16.7 mM glucose, as compared to the secretion at 1.7 mM glucose (Fig. 2). The islets cultured in the presence of the different concentrations of AG also responded similarly to glucose. Islet DNA and insulin contents were not affected by AG after the 48-hr culture (data not shown).

Culture of Islets for 6 Days with AG

In the next series of experiments, the effects of a more prolonged period of islet exposure to AG were studied. The islets were cultured with AG (0.1, 0.5, or 1.0 mM) for 6 days and at 2 different glucose concentrations, 11.1 mM and 28 mM. These AG concentrations were selected based on previous reports suggesting that AG inhibits both iNOS,

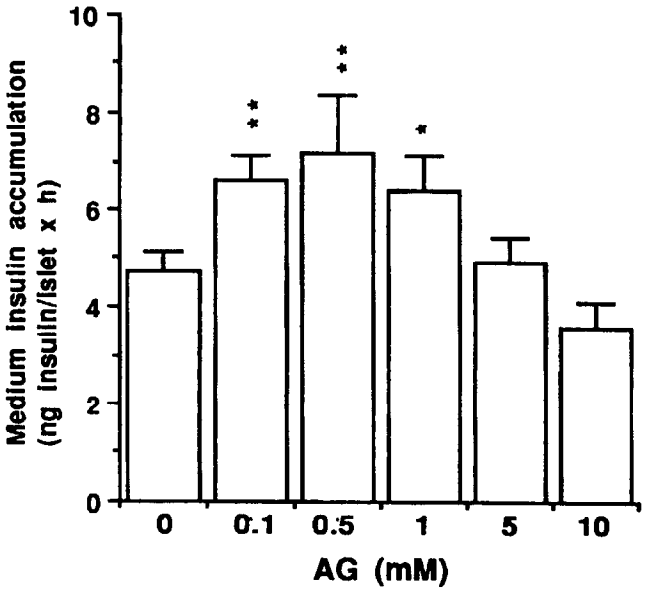

FIG. 1. Medium insulin accumulation from rat pancreatic islets cultured for 2 days in medium RPMI 1640 + 10% fetal calf serum in the absence or presence of aminoguanidine (AG), as indicated. Bars are means ± SEM for 12 experiments. * and ** denote $P < 0.05$ and $P < 0.01$ vs islets cultured in the absence of AG (Student's paired t-test).

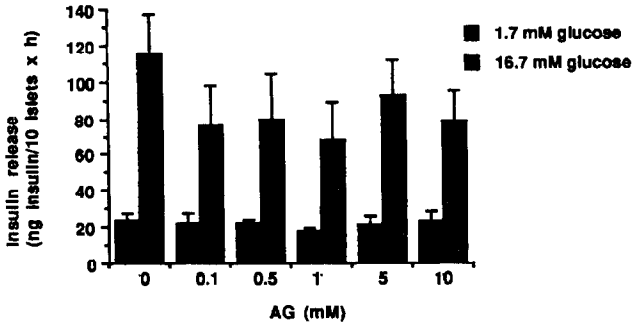

FIG. 2. Glucose-stimulated insulin release at 1.7 mM or 16.7 mM glucose from rat pancreatic islets cultured for 48 hr in medium RPMI 1640 + 10% fetal calf serum in the absence or presence of aminoguanidine (AG), as indicated. Bars are means ± SEM for 12 experiments.

in the range of 5–10 μM, and cNOS, at 0.5 mM and above [8, 27].

Islets cultured at 11.1 mM glucose with addition of AG showed a trend toward reduced DNA content, but the differences did not attain statistical significance (0.1 mM $P = 0.069$; 0.5 mM $P = 0.055$; 1.0 mM $P = 0.081$) (Table 1). This trend was not equally apparent in islets cultured at 28 mM glucose. AG did not affect insulin content, when expressed on a per islet basis, as compared to corresponding control islets. However, when expressing insulin content per DNA, islets cultured at 11.1 mM glucose in the presence of 0.1 mM AG had an increased insulin content. Medium insulin accumulation during the six-day culture was affected neither at 11.1 mM glucose (Fig. 3A) nor at 28 mM glucose (Fig. 3B) by any of the concentrations of AG tested. Glucose-stimulated insulin release after the 6-day culture was essentially unchanged both in islets cultured at 11.1 mM and at 28 mM glucose (Fig. 4). There was a slight decrease in basal insulin release in islets cultured at 11.1 mM glucose with the addition of 1.0 mM AG. The islet glucose oxidation rate was decreased 15–25% by 0.1–1.0 mM AG in islets cultured at 28 mM glucose, whereas no such effect was observed in islets cultured at 11.1 mM glucose (Table 2).

Finally, we tested whether or not exposure of islets to an even higher concentration of AG (5 mM) for up to 8 days would influence β-cell function. Insulin secretion at 16.7 mM glucose (ng insulin/10 islets) was inhibited both in islets cultured at 11.1 mM glucose (Control: 96.4 ± 15.0 vs AG: 48.5 ± 7.6; $P < 0.01$, ANOVA) and at 28 mM glucose (Control: 186 ± 8.5 vs AG: 96.7 ± 11.9; $P < 0.001$, ANOVA). However, AG did not alter islet DNA content (data not shown), which suggests that the effect was not due to AG-induced toxicity and cell death.

Blood Flow Measurements in Rats

We also examined the effect of AG (1, 10, and 50 mg/kg body weight) *in vivo* on pancreatic and islet blood flow in rats (Table 3). AG (50 mg/kg body weight) administration has previously been shown to increase mean arterial blood

TABLE 1. Long-term effects of aminoguanidine (AG) on DNA and insulin contents of rat pancreatic islets

Glucose (mM)	AG (mM)	DNA content (μg DNA/10 islets)	Insulin content (ng insulin/10 islets)	Insulin/DNA (ng insulin/μg DNA)
11.1	0	0.53 ± 0.08	652 ± 102	1364 ± 276
11.1	0.1	0.38 ± 0.07	706 ± 125	2070 ± 377*
11.1	0.5	0.35 ± 0.04	512 ± 57.7	1558 ± 268
11.1	1.0	0.38 ± 0.08	601 ± 82.3	1952 ± 490
28	0	0.47 ± 0.08	490 ± 109	1158 ± 279
28	0.1	0.40 ± 0.06	405 ± 68.7	1098 ± 221
28	0.5	0.47 ± 0.06	429 ± 104	1140 ± 338
28	1.0	0.40 ± 0.04	440 ± 70.5	1161 ± 246

The islets had been cultured for 6 days in medium RPMI 1640 + 10% FCS at either 11.1 mM or 28 mM glucose in the presence of AG, as indicated. Medium was changed every second day. Values are means ± SEM; $n = 5$. * denotes $P < 0.05$ compared to islets cultured in medium containing 11.1 mM glucose alone, using Student's paired *t*-test.

pressure by 10–15% in rats [7]. In the present study, mean arterial blood pressure was increased about 10% throughout the 30–35-min period after injection of 50 mg/kg body weight AG, but no significant effect was observed at lower doses of AG compared to saline-injected rats (data not shown). All doses of AG caused a decline in pancreatic blood flow. Islet blood flow was also decreased after administration of 50 mg/kg body weight of AG, while no such effect was observed after injection of lower doses. The fraction of the whole pancreatic blood flow diverted through the islets (i.e. ≈ 10%) remained unaffected by the 3 doses of AG.

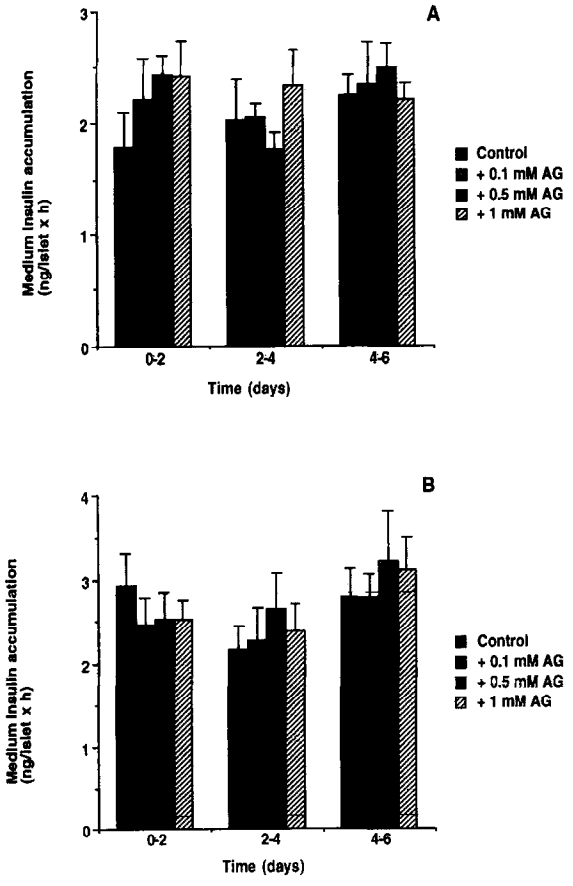

FIG. 3. Medium insulin accumulation from rat pancreatic islets cultured for 6 days in medium RPMI 1640 + 10% fetal calf serum at either 11.1 mM glucose (A) or at 28 mM glucose (B), in the absence or presence of aminoguanidine (AG), as indicated. Bars are means ± SEM for 5 experiments.

We subsequently examined the effect of AG (50 mg/kg body weight) on glucose-induced increase in islet blood flow. Thus, glucose (1 g/kg body weight) was injected intravenously 30 min after AG or saline injection and, 5 min later, the blood flows were measured. The animals injected with saline + glucose or AG + glucose were clearly hyperglycemic (≈ 18–20 mM) at the time of microsphere injection (data not shown). Glucose alone did not affect pancreatic blood flow (Table 3). However, when glucose was injected after AG, it restored AG-induced reduction in pancreatic blood flow to the level found in rats treated with saline alone. Glucose caused an increase in islet blood flow, which was also manifest in animals pretreated with AG injection.

DISCUSSION

The present study shows that prolonged *in vitro* exposure (2–6 days) of rat pancreatic islets to AG at concentrations that inhibit iNOS (≤0.5 mM) and cNOS (≥0.5 mM) [8, 27] was not toxic to the islet cells. Moreover, the data suggest that insulin production was not affected. Because previous data suggest that glucose may modify a putative stressful action on islet β-cells *in vitro* [17–19], and because the role of NO in regulation of islet β-cell insulin secretion has been debated [28–31], we performed experiments on islets cultured both at 11.1 mM glucose and 28 mM glucose in the presence or absence of AG. However, AG was not tested at lower glucose concentrations because it is well established that culture in medium RPMI 1640 at lower glucose concentrations (e.g. 5.6 mM) impairs rodent β-cell function [17, 32]. Basal NO production in cultured rat islets in the absence of exogenously added cytokines is very low [33, 34], and it is, therefore, likely that any changes in islet NO production induced by AG addition in the present

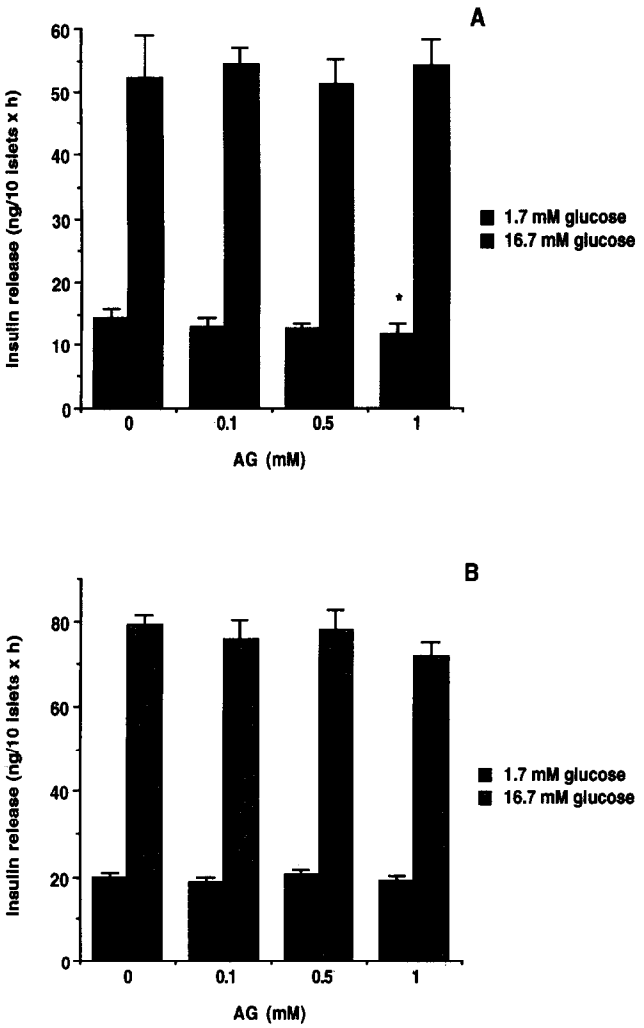

FIG. 4. Glucose-stimulated insulin release at 1.7 mM or 16.7 mM glucose from rat pancreatic islets cultured for 6 days in medium RPMI 1640 + 10% fetal calf serum at either 11.1 mM glucose (A) or at 28 mM glucose (B), in the absence or presence of aminoguanidine (AG), as indicated. Bars are means ± SEM for 5 experiments. * denotes $P < 0.05$ vs islets cultured in the absence of AG (Student's paired *t*-test).

study must have been very small. In line with this, we did not observe a modifying action of 28 mM glucose on the effect of AG addition. This may lend support to the notion that NO formation is not a prominent component in glucose-regulated insulin secretion from β-cells. On the other hand, when the islets were exposed to 5 mM AG for 8 days, islet glucose-stimulated insulin secretion was impaired. This is in agreement with a previous investigation, where the response to 16.7 mM glucose was reduced by >50% in rat islets cultured for 48 hr with addition of either 4.55 mM or 9.1 mM AG [35]. It is unlikely that this inhibitory action caused by a high concentration of AG may be attributed to NOS inhibition. It has, instead, been suggested that AG may cause a lowering of intracellular pH [36], and such an effect is likely to decrease islet insulin release [37, 38].

NO is probably the most important endothelial-derived relaxing factor present in the body, and its presence is necessary to maintain a normal tissue blood flow [39]. When AG was administered at a low dose, pancreatic blood flow declined, whereas islet blood flow remained unchanged. This is likely to reflect local regulatory mechanisms acting to maintain a sufficient islet blood flow in a situation of decrease in the pancreatic blood perfusion, here induced by an incomplete inhibition of NOS. A similar maintenance of islet blood flow has also been seen in other contexts with a decreased whole pancreatic blood perfusion [40, 41]. The administration of the NOS inhibitor AG at a higher dose caused a pronounced decrease of both whole pancreatic and islet blood flow, probably reflecting a more complete inhibition of the enzyme. Previous experiments with another NOS inhibitor, N^G-nitro-L-arginine, demonstrated a marked decrease in pancreatic blood flow, but an even more pronounced decline in islet blood perfusion [42]. This led to a decrease in fractional islet blood flow from 10 to 5% [42]. In the present study, however, no change in fractional islet blood flow could be observed. The reason for this discrepancy probably reflects the differences in sensitivity of iNOS and cNOS to the inhibitors used. AG is considered to selectively inhibit the inducible forms of NOS [7–9], whereas N^G-nitro-L-arginine affects both cNOS and iNOS

TABLE 2. Effects of aminoguanidine (AG) on the glucose oxidation rate of rat pancreatic islets maintained in culture at either 11.1 mM or 28 mM glucose

AG (mM)	Glucose oxidation rate (pmol/10 islets × 90 min)	
	11.1 mM glucose	28 mM glucose
0	648 ± 97.8	734 ± 62.2
0.1	612 ± 56.2	610 ± 55.3*
0.5	479 ± 54.8	549 ± 39.5†
1	657 ± 42.6	585 ± 40.4†

Values are means ± SEM; $n = 5$. * denotes $P < 0.01$ compared to corresponding islets not exposed to AG, paired *t*-test. † denotes $P < 0.05$ compared to corresponding islets not exposed to AG, paired *t*-test.

TABLE 3. Effects of saline, aminoguanidine (AG), or glucose (1 g/kg body weight) on pancreatic blood flow (PBF) and islet blood flow (IBF) in rats

Treatment	PBF (μL/min × g pancreas)	IBF (μL/min × g pancreas)	IBF/PBF (%)
Saline	467 ± 35	37.6 ± 4.0	8.2 ± 0.7
1 mg/kg BW AG	380 ± 25*	36.8 ± 1.8	9.9 ± 0.8
10 mg/kg BW AG	368 ± 27*	35.8 ± 3.7	9.8 ± 0.7
50 mg/kg BW AG	223 ± 28†	22.3 ± 3.6†	10.4 ± 1.7
Glucose	470 ± 30	62.7 ± 4.6†	13.5 ± 0.8§
Glucose + 50 mg/kg BW AG	491 ± 60¶	74.2 ± 6.1†‖	15.5 ± 1.4†,¶

Values are means ± SEM; $n = 6–7$. * denotes $P < 0.05$ compared to saline injected rats, ANOVA. † denotes $P < 0.001$ compared to saline injected rats, ANOVA. § denotes $P < 0.01$ compared to saline injected rats, ANOVA. ‖ denotes $P < 0.001$ compared to AG (50 mg/kg BW) injected rats, ANOVA. ¶ denotes $P < 0.01$ compared to AG (50 mg/kg BW) injected rats, ANOVA.

[9]. It has recently been demonstrated that both forms of the enzyme are present in rodent islets [43]. The present and previous findings are, therefore, compatible with the view that NO formed from both iNOS and cNOS can affect pancreatic and islet blood flow.

Glucose induced an increase in islet blood flow in the control rats, as described previously [25, 44]. This increase was unaffected by pretreatment with AG, but was previously shown to be abolished by inhibition of cNOS [42]. It can be assumed that a prerequisite for the high basal islet blood perfusion, 10 times higher than that of the exocrine pancreas [25], is that a basal production of NO occurs within the islet vasculature. This is likely to take place by the action of cNOS but, under certain conditions, a contribution of NO generation from iNOS activity might occur. If one or both of these enzymes are inhibited, a decreased blood perfusion would occur, because vasoconstrictors are allowed a more unantagonized action on islet vasculature. When glucose is administered, an increased vagal neural input to the islets occurs, which also mediates the islet blood flow increase [42]. It seems as if vagal stimulation of islet blood perfusion can take place, even though only NO formed from cNOS is present within the islets. It is, therefore, possible that NO produced by cNOS is needed to maintain high islet blood flow, whereas NO formed by iNOS can modulate islet blood flow. In the latter case, it is likely that NO exerts its major physiological effects on islets by other means than by acting on islet blood perfusion.

In conclusion, the present *in vitro* data suggest that prolonged exposure to AG at concentrations known to inhibit iNOS was not harmful to β-cells. However, at high concentrations of AG, which might be required to prevent nonenymatic glycation of proteins related to diabetes complications, β-cell function might be inhibited. In this context, it is of interest that a high concentration of AG (5 mM) also inhibited insulin output from human pancreatic islets *in vitro* [45]. *In vivo*, the compound decreased the pancreatic blood perfusion of rats, an effect which, if sustained, might have consequences for both the exocrine and endocrine function of the gland.

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References

1. Corbett JA and McDaniel ML, Does nitric oxide mediate autoimmune destruction of β-cells? Possible therapeutic interventions in IDDM. *Diabetes* **41:** 897–903, 1992.
2. Kolb H and Kolb-Bachofen V, Nitric oxide: a pathogenetic factor in autoimmunity. *Immunol Today* **13:** 157–160, 1992.
3. Lowenstein CJ and Snyder SH, Nitric oxide, a novel biologic messenger. *Cell* **70:** 705–707, 1992.
4. Prince RC and Gunson DE, Rising interest in nitric oxide synthase. *Trends Biochem Sci* **18:** 35–36, 1993.
5. Lukic ML, Stosic-Grujicic S, Ostojic N, Chan WL and Liew FY, Inhibition of nitric oxide generation affects the induction of diabetes by streptozocin in mice. *Biochem Biophys Res Commun* **178:** 913–920, 1991.
6. Kolb H, Kiesel U, Kröncke K-D and Kolb-Bachofen V, Suppression of low dose streptozotocin induced diabetes in mice by administration of a nitric oxide synthase inhibitor. *Life Sci* **49:** PL213–PL-217, 1991.
7. Corbett JA, Tilton RG, Chang K, Hasan KS, Ido Y, Wang JL, Sweetland MA, Lancaster Jr JR, Williamsson JR and McDaniel ML, Aminoguanidine, a novel inhibitor of nitric oxide formation, prevents diabetes vascular dysfunction. *Diabetes* **41:** 552–556, 1992.
8. Misko TP, Moore WM, Kasten TP, Nickols GA, Corbett JA, Tilton RG, McDaniel ML, Williamsson JR and Currie MG, Selective inhibition of the inducible nitric oxide synthase by aminoguanidine. *Eur J Pharmacol* **233:** 119–126, 1993.
9. Joly GA, Ayres M, Chelly F and Kilbourn RG, Effects of N^G-methyl-L-arginine, N^G-nitro-L-arginine, and aminoguanidine on constitutive and inducible nitric oxide synthase in rat aorta. *Biochem Biophys Res Commun* **199:** 147–154, 1994.
10. Tilton RG, Chang K, Corbett JA, Misko TP, Currie MG, Bora NS, Kaplan HJ and Williamsson JR, Endotoxin-induced uveitis in the rat is attenuated by inhibition of nitric oxide production. *Invest Ophthalmol Vis Sci* **35:** 3278–3288, 1994.
11. Cross AH, Misko TP, Lin RF, Hickey WF, Trotter JL and Tilton RG, Aminoguanidine, an inhibitor of inducible nitric oxide synthase, ameliorates experimental autoimmune encephalomyelitis in SJL mice. *J Clin Invest* **93:** 2684–2690, 1994.
12. Brownlee M, Vlassara H, Kooney A, Ulrich P and Cerami A, Aminoguanidine prevents diabetes-induced arterial wall protein cross-linking. *Science* **232:** 1629–1632, 1986.
13. Makita Z, Bucala R, Rayfield EJ, Friedman EA, Kaufman AM, Korbet SM, Barth RH, Winston JA, Fuh H, Manogue KR, Cerami A and Vlassara H, Reactive glycosylation endproducts in diabetic uraemia and treatment of renal failure. *Lancet* **343:** 1519–1522, 1994.
14. Corbett JA, Mikhael A, Shimizu J, Frederick K, Misko TP, McDaniel ML, Kanagawa O and Unanue ER, Nitric oxide production in islets from nonobese diabetic mice: aminoguanidine-sensitive and -resistant stages in the immunological diabetic process. *Proc Natl Acad Sci USA* **90:** 8992–8995, 1993.
15. Wu G, Nitric oxide synthesis and the effect of aminoguanidine and N^G-monomethyl-L-arginine on the onset of diabetes in the spontaneously diabetic BB rat. *Diabetes* **44:** 360–364, 1995.
16. Holstad M and Sandler S, Aminoguanidine, an inhibitor of nitric oxide formation, fails to protect against insulitis and hyperglycemia induced by multiple low dose streptozotocin injections in mice. *Autoimmunity* **15:** 311–314, 1993.
17. Sandler S and Andersson A, Cryopreservation of mouse pancreatic islets: effects of different glucose concentrations in the post-thaw culture medium on islet recovery. *Cryobiology* **24:** 285–291, 1987.
18. Eizirik DL, Strandell E and Sandler S, Culture of mouse pancreatic islets in different glucose concentrations modifies B cell sensitivity to streptozotocin. *Diabetologia* **31:** 168–174, 1988.
19. Sandler S, Bendtzen K, Eizirik DL, Strandell E, Welsh M and Welsh N, Metabolism and β-cell function of rat pancreatic islets exposed to human interleukin-1β in the presence of a high glucose concentration. *Immunol Lett* **26:** 245–252, 1990.
20. Sandler S, Andersson A and Hellerström C, Inhibitory effects of interleukin 1 on insulin secretion, insulin biosynthesis, and oxidative metabolism of rat pancreatic islets. *Endocrinology* **121:** 1424–1431, 1987.

21. Heding LG, Determination of total serum insulin (IRI) in insulin-treated patients. *Diabetologia* **8:** 260–266, 1972.
22. Krebs HA and Henseleit K, Untersuchungen über die Harnstoffbildung im Tierkörper. *Hoppe-Seylers Z Physiol Chem* **210:** 33–66, 1932.
23. Hinegardner RT, An improved fluorometric assay for DNA. *Anal Biochem* **39:** 197–201, 1971.
24. Andersson A and Sandler S, Viability tests of cryopreserved endocrine pancreatic cells. *Cryobiology* **20:** 161–168, 1983.
25. Jansson L and Hellerström C, Stimulation by glucose of the blood flow to the pancreatic islets of the rat. *Diabetologia* **25:** 45–50, 1983.
26. Jansson L and Hellerström C, A rapid method of visualizing the pancreatic islets for studies of islet capillary blood flow using non-radioactive microspheres. *Acta Physiol Scand* **113:** 371–374, 1981.
27. Wolff DJ and Lubeskie A, Aminoguanidine is an isoform-selective, mechanism-based inactivator of nitric oxide synthase. *Arch Biochem Biophys* **316:** 290–301, 1995.
28. Laychock SG, Modica ME and Cavanaugh CT, L-arginine stimulates cyclic guanosine 3′,5′-monophosphate formation in rat islets of Langerhans and RINm5F insulinoma cells: evidence for L-arginine: nitric oxide synthase. *Endocrinology* **129:** 3043–3052, 1991.
29. Schmidt HHHW, Warner TD, Ishii K, Sheng H and Murad F, Insulin secretion from pancreatic B cells caused by L-arginine-derived nitrogen oxides. *Science* **255:**721–723, 1992.
30. Jones PM, Persaud SJ, Bjaaland T, Pearson JD and Howell SL, Nitric oxide is not involved in the initiation of insulin secretion from rat islets of Langerhans. *Diabetologia* **35:** 1020-1027, 1992.
31. Vincent SR, Nitric oxide and arginine-evoked insulin secretion. *Science* **258:** 1376, 1992.
32. Svensson C and Hellerström C, Long-term effects of a high glucose concentration in vitro on the oxidative metabolism and insulin production of isolated rat pancreatic islets. *Metabolism* **40:** 513–518, 1991.
33. Southern C, Schulster D and Green IC, Inhibition of insulin secretion by interleukin-1β and tumour necrosis factor-α via an L-arginine-dependent nitric oxide generating mechanism. *FEBS Lett* **276:** 42–44, 1990.
34. Welsh N, Eizirik DL, Bendtzen K and Sandler S, Interleukin-1β-induced nitric oxide production in isolated rat pancreatic islets requires gene transcription and may lead to inhibition of the Krebs cycle enzyme aconitase. *Endocrinology* **129:** 3167–3173, 1991.
35. Tasaka Y, Nakaya F, Matsumoto H and Omori Y, Effects of aminoguanidine on insulin release from pancreatic islets. *Endocrine J* **41:** 309–313, 1994.
36. Zähner D and Malaisse WJ, Effects of advanced glycation products and aminoguanidine upon insulin release. *Diab Nutr Metab* **5:** 43–46, 1992.
37. Hutton JC, Sener A, Herchuelz A, Valverde I, Boscher AC and Malaisse WJ, The stimulus secretion coupling of glucose-induced insulin release, XLII. Effects of extracellular pH on insulin release: their dependency on nutrient concentration. *Horm Metab Res* **12:** 294–299, 1980.
38. Arkhammar P, Berggren P-O and Rorsman P, Direct evidence for opposite effects of D-glucose and D-glyceraldehyde on cytoplasmic pH of mouse pancreatic β-cells. *Biosci Rep* **6:** 355–361, 1986.
39. Palmer RMJ, Ferrige AG and Moncada S, Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* **327:** 524–526, 1987.
40. Jansson L, Whole pancreatic blood flow and islet blood flow in hypovolemic hypotension in rats. *Eur Surg Res* **24:** 291–297, 1992.
41. Hindlycke M and Jansson L, Isovolemic hemodilution and pancreatic islet blood flow in the rat. *Int Surg* **78:** 254–256, 1993.
42. Svensson AM, Östenson C-G, Sandler S, Efendic S and Jansson L, Inhibition of nitric oxide synthase by N^G-nitro-L-arginine causes preferential decrease in pancreatic islet blood flow in normal rats and spontaneously diabetic GK rats. *Endocrinology* **135:** 849–853, 1994.
43. Suscheck C, Fehsel K, Kröncke K-D, Sommer A and Kolb-Bachofen V, Primary culture of rat islet capillary endothelial cells—constitutive and cytokine-inducible macrophagelike nitric oxide synthases are expressed and activities regulated by glucose concentration. *Am J Pathol* **145:** 685–695, 1994.
44. Jansson L and Hellerström C, Glucose-induced changes in pancreatic islet blood flow mediated by central nervous system. *Am J Physiol* **251:** E644–E647, 1986.
45. Eizirik DL, Sandler S, Welsh N, Cetkovic-Cvrlje M, Niemann A, Geller DA, Pipeleers DG, Bendtzen K and Hellerström C. Cytokines supress human islet functions irrespective of their effects on nitric oxide generation. *J Clin Invest* **93:** 1968–1974, 1994.