Effect of Poly(ADP-ribose) Synthetase Inhibitor Administration to Rats Before and After Injection of Alloxan and Streptozotocin on Islet Proinsulin Synthesis

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SUMMARY

Nicotinamide (10 mmol/kg) and 3-aminobenzamide (1.25 mmol/kg), poly(ADP-ribose) synthetase inhibitors, were injected intravenously to rats either 30 min before the intravenous administration of 12 mg/kg alloxan or 50 mg/kg streptozotocin ("pretreatment") or 5 min after the administration ("posttreatment"). Fifteen minutes after the injection of the diabetogenic agents, pancreatic islets were isolated from the rats and proinsulin synthesis was determined. Proinsulin synthesis was decreased in islets from rats treated with alloxan or streptozotocin. Pretreatment with poly(ADP-ribose) synthetase inhibitors was found to protect against alloxan- or streptozotocin-induced decrease in proinsulin synthesis. By posttreatment with poly(ADP-ribose) synthetase inhibitors, streptozotocin-induced decrease in proinsulin synthesis was also significantly reversed, whereas the decrease induced by alloxan was not.

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Alloxan and streptozotocin produce diabetes mellitus in experimental animals. The diabetogenic agents have been known to inhibit various islet cell functions including proinsulin synthesis.[1–3] Our recent study has clarified how the diabetogenic agents inhibit islet proinsulin synthesis; both alloxan and streptozotocin cause DNA strand breaks to activate nuclear poly(ADP-ribose) synthetase, thereby depleting intracellular NAD level and inhibiting proinsulin synthesis.[4–7] This raises the possibility that alloxan- and streptozotocin-induced diabetes may be preventable by inhibiting islet poly(ADP-ribose) synthetase.

The present study was performed to determine if an intravenous administration of poly(ADP-ribose) synthetase inhibitors would prevent the inhibition of islet proinsulin synthesis in rats injected with alloxan or streptozotocin. Administration of poly(ADP-ribose) synthetase inhibitors before alloxan or streptozotocin injection was found to prevent the inhibition of proinsulin synthesis caused by the diabetogenic agents. On the other hand, the administration after alloxan or streptozotocin injection was found to protect against streptozotocin-induced inhibition of proinsulin synthesis, but to fail in protecting against alloxan-induced inhibition of proinsulin synthesis.

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MATERIALS AND METHODS

Alloxan monohydrate and nicotinamide were purchased from Wako Pure Chemical Industries (Osaka, Japan), streptozotocin from Sigma (St. Louis, Missouri), and L-[^{3}H]leucine (112 Ci/mmol) from New England Nuclear (Boston, Massachusetts). 3-Aminobenzamide hydrochloride was synthesized in Research Laboratories (Chugai Pharmaceutical Co., Ltd., Tokyo, Japan). Male Wistar rats weighing 150–200 g were fed ad libitum and used for this study.

Alloxan and streptozotocin were dissolved in saline just before use and injected via the tail vein of ether-anesthetized rats in doses of 12 mg/kg and 50 mg/kg, respectively. Nicotinamide (10 mmol/kg) and 3-aminobenzamide (1.25 mmol/kg) were injected intravenously either 30 min before the administration of alloxan or streptozotocin ("pretreatment") or 5 min after the administration ("posttreatment"). Fifteen minutes after the injection of the diabetogenic agent, the pancreas was removed and islets of Langerhans were isolated by the collagenase digestion method as described previously.[8] Batches of 30 islets were incubated at 37°C for 60 min in 100 μl of Krebs-Ringer bicarbonate medium containing 4, 10, and 20 mM glucose and 10 μCi of [^{3}H]leucine.[7] Proinsulin synthesized was determined as described previously.[9]

Results are expressed as mean ± SD, and differences between rats treated with and without poly(ADP-ribose) synthetase inhibitors are evaluated by Student's one-tailed, paired *t* test.

RESULTS

Ten millimoles per kilogram of nicotinamide or 1.25 mmol/kg 3-aminobenzamide, islet poly(ADP-ribose) synthetase inhibitors,[7,10] was injected 30 min before the intravenous administration of alloxan or streptozotocin ("pretreatment"). Fifteen minutes after the diabetogenic agent was injected, pancreatic islets were isolated and proinsulin synthesis was examined. In islets from control rats, proinsulin synthesis was greatly stimulated with increasing concentrations of glucose. As shown in Figure 1*a*, 12 mg/kg alloxan decreased proinsulin synthesis to 36%, 33%, and 31% of the control at 4, 10, and 20 mM glucose, respectively. Pretreatment with nicotinamide was found to significantly reverse the decrease to 92%, 91%, and 106% at 4, 10, and 20 mM glucose ($P < 0.005$–0.05 versus without pretreatment). Pretreatment with 3-aminobenzamide also resulted in a significant reversal of the alloxan-induced decrease in islet proinsulin synthesis (Figure 1*b*). Streptozotocin-induced inhibition of proinsulin synthesis (30–35% of the control) was also almost completely prevented by the pretreatment with either nicotinamide (74–106%) or 3-aminobenzamide (77–85%) (Figures 1*c* and *d*). Administration of poly(ADP-ribose) synthetase inhibitors alone did not affect proinsulin synthesis at the doses used (data not shown).

Next, nicotinamide or 3-aminobenzamide was injected 5 min after the diabetogenic agents (Figure 2). As shown in Figures 2*c* and *d*, streptozotocin-induced decrease in proinsulin synthesis was reversed by the posttreatment with either nicotinamide or 3-aminobenzamide ($P < 0.005$–0.05 versus

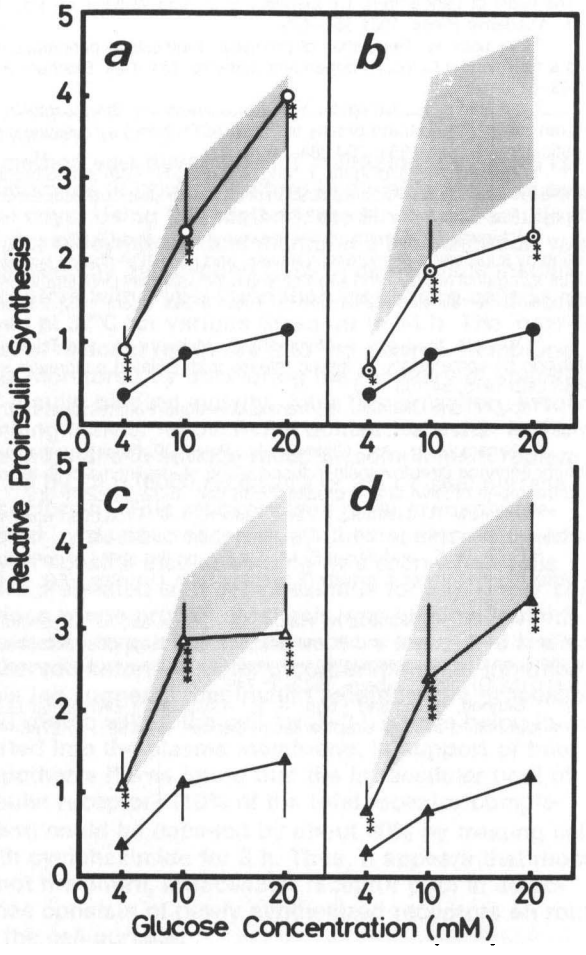

FIGURE 1. Effect of pretreatment with nicotinamide or 3-aminobenzamide on alloxan- or streptozotocin-induced inhibition of islet proinsulin synthesis. Each point (mean ± SD, N = 5) was related to the control value at 4 mM glucose, which corresponded to 762.3 ± 50.4 cpm/60 min/islet (N = 20). Shaded area shows mean ± SD of the control. Control rats were treated with saline. ●, proinsulin synthesis in islets from rats treated with 12 mg/kg alloxan; ○, with 12 mg/kg alloxan and 10 mmol/kg nicotinamide (*a*); ⊙, with 12 mg/kg alloxan and 1.25 mmol/kg 3-aminobenzamide (*b*); ▲, with 50 mg/kg streptozotocin; △, with 50 mg/kg streptozotocin and 10 mmol/kg nicotinamide (c); ◬, with 50 mg/kg streptozotocin and 1.25 mmol/kg 3-aminobenzamide (*d*). The concentration of glucose added is shown on the abscissa. *, **, *, and ****, $P < 0.05$, 0.025, 0.01, and 0.005 versus alloxan- or streptozotocin-treated rats without pretreatment.**

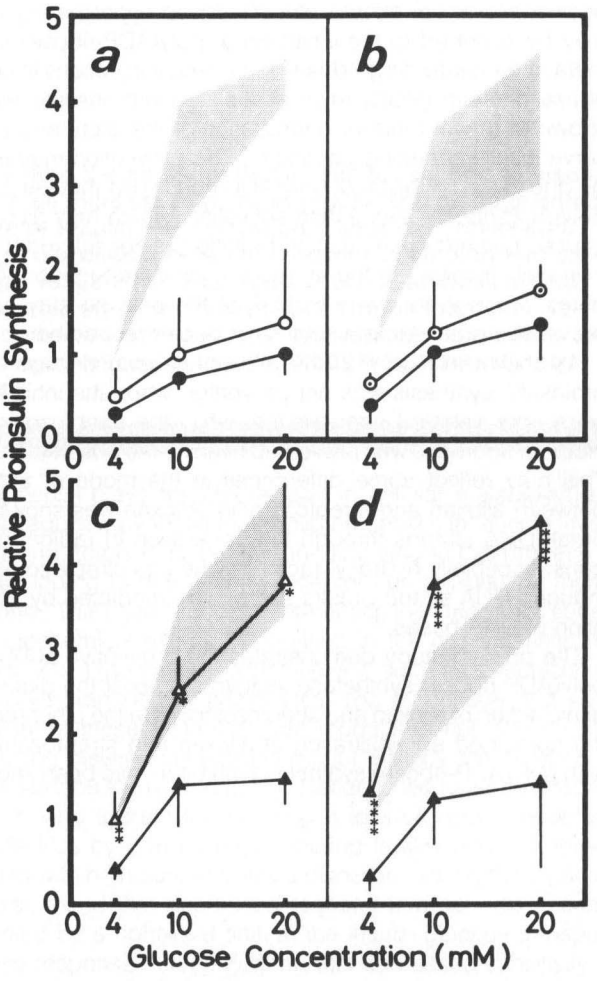

FIGURE 2. Effect of posttreatment with nicotinamide or 3-aminobenzamide on alloxan- or streptozotocin-induced inhibition of islet proinsulin synthesis. Each point (mean ± SD) was related to the control value at 4 mM glucose, which corresponded to 586.5 ± 39.9 cpm/60 min/islet (N = 19). Each experiment was carried out five times except for that with streptozotocin and nicotinamide (N = 4). Shaded area shows mean ± SD of the control. Control rats were treated with saline. ●, proinsulin synthesis in islets from rats treated with 12 mg/kg alloxan; ○, with 12 mg/kg alloxan and 10 mmol/kg nicotinamide (*a*); ⊙, 12 mg/kg alloxan and 1.25 mmol/kg 3-aminobenzamide (*b*); ▲, with 50 mg/kg streptozotocin; △, 50 mg/kg streptozotocin and 10 mmol/kg nicotinamide (c); ◬, 50 mg/kg streptozotocin and 1.25 mmol/kg 3-aminobenzamide (*d*). The concentration of glucose added is shown on the abscissa. *, **, *, and ****, $P < 0.05$, 0.025, 0.01, and 0.005 versus alloxan- or streptozotocin-treated rats without posttreatment.**

without posttreatment). However, the alloxan-induced decrease was not prevented by the posttreatment (Figures 2*a* and *b*).

DISCUSSION

The present study has clearly demonstrated that pretreatment with nicotinamide or 3-aminobenzamide, poly(ADP-ribose) synthetase inhibitors, protected against alloxan- and streptozotocin-induced decrease in islet proinsulin synthesis. Posttreatment with poly(ADP-ribose) synthetase inhibitors also protected against streptozotocin-induced decrease in proinsulin synthesis but failed in protecting against the alloxan-induced decrease. Poly(ADP-ribose) synthetase inhibitors are considered to preserve islet proinsulin synthesis by inhibiting NAD degradation through poly(ADP-ribose), as we have proposed.[4–7] The protecting effect of nicotinamide against alloxan- or streptozotocin-induced hyperglycemia[11,12] may be ascribed to the inhibition of poly(ADP-ribose) synthetase. Recently, large-dose nicotinamide injections in nonobese diabetic (NOD) mice associated with insulitis were shown to prevent further degradation of the islets and preserve insulin secreting capacity.[13] The prevention may also be due to the inhibition of poly(ADP-ribose) synthetase. Islet poly(ADP-ribose) synthetase activation, which may also be caused by certain viruses and radiation, is considered to be intimately involved in the etiology of insulin-dependent diabetes,[4,5] and therefore, inhibitors of the enzyme may have preventive potential against insulin-dependent diabetes.

As shown in Figure 2, the alloxan-induced decrease in proinsulin synthesis was not prevented when the inhibitors were administrated after alloxan, while the streptozotocin-induced decrease was prevented even by the posttreatment. This may reflect some differences in the mode of action between alloxan and streptozotocin. Alloxan was shown to break DNA strands through the generation of radical oxygens, especially hydroxyl radical,[7] whereas streptozotocin-induced DNA strand breaks seem to be mediated by alkylation of DNA bases.[14]

The present study demonstrates the preventive ability of poly(ADP-ribose) synthetase inhibitors against the diabetogenic action of alloxan and streptozotocin. On the other hand, the combined administration of alloxan and streptozotocin with poly(ADP-ribose) synthetase inhibitors has been known to induce islet cell tumors.[4] The mechanism of the tumorigenesis is now under investigation in our laboratory.

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