NOTE

Effects of Aminoguanidine on Glucagon and Insulin Release from Rat Pancreatic Islet

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Abstract. Aminoguanidine (AG) is a potential therapeutic agent for preventing the generation of advanced glycation end products in diabetes mellitus. In this study, the effects of AG on glucagon and insulin secretion in in vitro rat pancreatic islets were investigated. The islets were aseptically isolated and cultured in tissue culture medium 199 for 48 h with or without 9.1 mM AG (1 mg/ml). After the culture, 50 islets were perifused in Krebs-Ringer bicarbonate buffer containing 20 mM arginine or 1 U/ml pancreozymin in the presence of 3.3 mM glucose. Islets previously exposed to AG showed similar glucagon response to control islets at a 20 mM arginine concentration, and insulin response, too. Glucagon release caused by 1 U/ml pancreozymin from the islets previously exposed to AG was also not different from that of the control islets, but the release of insulin was much lower than that of control. These results suggest that AG would not be toxic to α-cells but toxic to β-cells at high concentrations, although there is slightly different sensitivity to β-cell secretagogues.

Key words: Aminoguanidine, Glucagon, Insulin, Pancreatic islets

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Materials and Methods

Materials

AG was supplied by Wako Pure Chemical Industries Ltd. (Osaka, Japan) and pancreozymin by Sigma Chemical Co. (St. Louis, USA). Heat-inactivated bovine calf serum and penicillin-streptomycin were obtained from Gibco (N.Y., USA).

Islet culture

Pancreatic islets were aseptically isolated by a modification of the method of Lacy and Kostianovsky [10, 11]. The islets were maintained in 35 mm Corning-culture plastic flasks (Iwaki glass, Tokyo, Japan) in tissue culture medium 199 (glucose concentration 5.5 mM) supplemented with 10% heat-inactivated fetal bovine serum and 1% penicillin-streptomycin. The islets were cultured...
at 37 °C for 48 h in a humidified atmosphere of 5% CO₂/95% air with or without AG added to the medium.

**Perifusion of isolated islets**

The cultured islets were centrifuged and washed once. For the perifusion experiment, batches of 50 islets were transferred to plastic filter chambers (Nihon Millipore Filter Kogyo K. K., Tokyo, Japan) fitted with nylon filters (8 μm pore size). The basic perifusion medium was Krebs-Ringer bicarbonate buffer containing 2 mg/ml BSA and 3.3 mM glucose. Aprotinine (250 U/ml) was added to protect against proteolytic degradation of glucagon. The preperifusion time was 45 min. The flow rate was 1 ml/min, effluent was collected at 1 min intervals, and samples were stored at −20 °C for further determination. Arginine was given as L-arginine hydrochloride.

**Assays**

IRG levels in the medium were determined with an IRG immunoassay kit supplied by Dainabot ltd. (Tokyo, Japan), and IRI levels were measured by the two-antibody immunoassay of Morgan and Lazarow [12] with a rat insulin standard. Results were expressed as the mean ± SEM. Statistical analysis was performed by paired Student’s t-test. Some of the results were compared by nonparametric Mann-Whitney U-test and P<0.05 was considered to indicate a significant difference.

**Results**

**Effects of AG on IRG and IRI release by arginine**

The effects of arginine on the secretion of IRG and IRI from the perifused islets were investigated. Before perifusion the islets were cultured for 48 h with or without 9.1 mM AG. The perifused islets showed a rapid IRG response to 20 mM arginine. The IRG level increased significantly from 8.21 ± 1.83 to 14.10 ± 2.21 fmol/ml at 3 min in the AG-exposed islets (P<0.0001) and from 10.31 ± 2.60 to 14.78 ± 2.75 fmol/ml in the control islets (P<0.0001). In IRI secretion, IRI release from AG-exposed and from non-exposed islets was also similar (the former basal level: 145 ± 47 and 1 min level: 274 ± 99 fmol/ml; the latter basal level: 207 ± 60 and 1 min level: 363 ± 90 fmol/ml, and the increases from the basal level are both significant: P<0.0002).

**Effects of AG on IRG and IRI release by pancreozymin**

The effects of pancreozymin on IRG and IRI release from AG-exposed or non-AG-exposed islets were investigated. Pancreozymin was infused for

![Fig. 1. Effects of arginine on the release of IRG from pancreatic islets. Pancreatic islets exposed to 9.1 mM AG were perifused for 20 min with 20 mM arginine in a medium of Krebs-Ringer bicarbonate buffer containing 2 mg/ml BSA and 3.3 mM glucose. The upper figure shows the results of an experiment on AG-exposed islets, and the lower one those for control islets.](image-url)
EFFECTS OF AMINOGUANIDINE ON GLUCAGON AND INSULIN

Fig. 2. Effects of arginine on the release of IRI from pancreatic islets. Pancreatic islets exposed to 9.1 mM AG were perfused for 20 min with 20 mM arginine in a medium of Krebs-Ringer bicarbonate buffer containing 2 mg/ml BSA and 3.3 mM glucose. The upper figure shows the results of an experiment of AG-exposed islets, and the lower one those for control islets.

Fig. 3. Effects of pancreozymin on the release of IRG from pancreatic islets. Pancreatic islets exposed to 9.1 mM AG were perfused for 10 min with 1 U/ml pancreozymin in a medium of Krebs-Ringer bicarbonate buffer containing 2 mg/ml BSA and 3.3 mM glucose. The upper figure shows the results of an experiment on AG-exposed islets, and the lower one those for control islets.

ten min at a concentration of 1 U/ml. Figure 3 shows the stimulatory effect of pancreozymin on IRG secretion. The mean IRG levels at the peak after pancreozymin infusion were 22.67 ± 4.68 fmol/ml at 8 min after starting the perfusion (basal value: 7.17 ± 2.09 fmol/ml) in the AG-exposed islets and 23.07 ± 6.60 fmol/ml at 10 min (basal value: 7.20 ± 2.41 fmol/ml) in the controls. The patterns of IRG response were similar to each other. Infusion of pancreozymin was also associated with a rise in IRI secretion from the control islets, but no significant rise in IRI secretion from AG-exposed islets was found (the peak level of former: 273 ± 63 fmol/ml at 9 min, the latter: 741 ± 119 fmol/ml, respectively).

Comparison of cumulative IRG and IRI release

Cumulative IRG and IRI release after arginine or pancreozymin were compared in AG-exposed and non-exposed islets (Table 1). The cumulative values for IRG release during 20 min infusion of arginine in the AG-exposed and non-exposed control islets were similar (P<0.89) and that of IRI release during 10 min infusion of pancreozymin was also similar (P<0.81), but IRI response was
different. Although the IRI release value during arginine infusion was not statistically significant in AG-exposed and control islets, during pancreozymin infusion it was much lower in the AG-exposed islets than in the non-exposed ones (P<0.03).

Discussion

We previously reported already that the islets exposed to AG 9.1 mM (1 mg/ml) release significantly less IRI in response to a high glucose concentration [9]. In this experiment, the IRG release from the islets exposed to AG 9.1 mM caused by 20 mM arginine or pancreozymin (1 U/ml) was not significantly different from that of the controls. Pancreozymin is a potent secretagogue of IRG, as already reported [13, 14], together with arginine [15, 16]. From these findings α-cells seem to be more resistant to AG than β-cells. On the other hand IRI release from the exposed islets was inhibited during infusion of pancreozymin just as with high glucose infusion [8, 9, 24]. In the case of pancreozymin infusion, the basal IRI release value before the start was slightly low, but similar to that of arginine infusion and the summed net increase in IRI was also small, but not significant (182.4 ± 87.8 vs. 112.5 ± 51.9). These findings present a striking contrast to those during arginine infusion experiment. In arginine infusion, the IRI release was not inhibited. Similar differential sensitivity to β-cell secretagogues has already been reported. Palmer et al. [16] reported that there was marked attenuation of the early β-cell response upon stimulation by glucose in diabetic subjects compared with normal controls, although the acute insulin response to arginine was normal. Ganda et al. [17] studied the insulin secretory response to various β-cell secretagogues in four children in early stages or remission of type 1 diabetes mellitus and found that the peak first-phase insulin increase

Table 1. Comparison of cumulative IRG and IRI release during perifusion with 20 mM arginine or with 1 U/ml pancreozymin

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<tr>
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<th>IRG</th>
<th>IRI</th>
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<tr>
<td></td>
<td>A</td>
<td>B</td>
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<tr>
<td>20 mM arginine</td>
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<tr>
<td>9.1 mM AG</td>
<td></td>
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<tr>
<td>Pre-expose</td>
<td>262 ± 31 fmol</td>
<td>4.76 ± 1.92 pmol</td>
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<tr>
<td>Control</td>
<td>255 ± 43</td>
<td>5.68 ± 1.52</td>
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<td>NS</td>
<td>(n=7)</td>
<td>(n=8)</td>
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<tr>
<td>1 U/ml pancreozymin</td>
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<tr>
<td>9.1 mM AG</td>
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<tr>
<td>Pre-expose</td>
<td>189 ± 35 fmol</td>
<td>2.25 ± 0.30 pmol</td>
</tr>
<tr>
<td>Control</td>
<td>204 ± 50</td>
<td>5.42 ± 0.89</td>
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<td>NS</td>
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after iv glucose was noticeably attenuated in each patient, whereas the peak response to iv arginine was similar to normal control subjects. The β-cell response to pancreozymin in the presence of AG was therefore considered to be more sensitive to its toxic effects than that in case of arginine. The generation of nitric oxide is associated with inhibition of glucose-induced insulin secretion [18]. AG is reported to be an inhibitor of nitric oxide synthase activity [5, 19]. The mechanism of the inhibitory effect of AG on pancreatic-β cell cannot be explained from the standpoint of the inhibition of nitric oxide synthase activity, although no effect of AG treatment on insulitis or diabetes onset in spontaneously developing diabetes in NOD mice or of treatment of the other nitric oxide synthase inhibitor in the pancreas perfusion experiment was found [20, 21]. Some toxic effects of AG may be taken into consideration. In nondiabetic rats given AG orally (50 mg/kg bw) once daily for at least 8 days serum levels of AG have been reported to be 8.5 µg/g [22]. From these data the concentration of AG in this experiment was high (around 100 times as high). Zähner and Malaisse [8] mentioned that AG may alter the intracellular pH and the change in intracellular pH inhibits IRI secretion [23]. On the other hand, the α-cells of the pancreatic islet remains intact in spite of the high concentration of AG. Thus the diverse effects of AG on the pancreatic islets should be considered.

References

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