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Enhancement by streptozotocin of O_2^- radical generation by the xanthine oxidase system of pancreatic β-cells

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Spin-trapping techniques and electron spin resonance (ESR) spectroscopy were used to study the relationship between the effect of streptozotocin (STZ) on pancreatic β-cells and free radical formation by these cells. Results showed that STZ enhanced generation of the DMPO-OH radical adduct, which is a degradation product of the superoxide anion (O_2^-) in the presence of cellular components, in a hypoxanthine-xanthine oxidase (XOD) system with a homogenate of β-cells. This enhancing effect was also observed in a system without cellular components; STZ increased the signal height due to the O_2^- radical in a concentration-dependent manner and caused a maximum of 150% enhancement at a concentration of 1.5 mM. Thus, STZ seemed to enhance the generation of the O_2^- radical in the XOD system, probably by some mechanism of its interaction with XOD. Pancreatic β-cells exhibited a high XOD activity and a very low superoxide dismutase activity. Therefore, the present result supports the possibility that the cytotoxic effect of STZ is closely related to free radical generation in pancreatic β-cells.

Xanthine oxidase; Superoxide dismutase; Superoxide radical; Streptozotocin; (Pancreatic β-cell)

1. INTRODUCTION

Streptozotocin (STZ) [1,2] and alloxan [3] show selective cytotoxicity on pancreatic β-cells, and thus cause insulin-dependent diabetes. Their mechanisms of action are unknown, but since their actions are inhibited by superoxide dismutase (SOD) [4,5] or a chemical scavenger, such as 1,1-dimethylurea [6], they have been suggested to be related to the formation of free radicals. In fact, some reports strongly support the participation of free radicals in the action of alloxan [7,8]. In this work, we investigated the relation between the action of STZ on β-cells and their generation of free radicals, measured by electron spin resonance (ESR) spectrometry.

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2. MATERIALS AND METHODS

SOD, XOD (type I), STZ, penicillin G and streptomycin sulfate were purchased from Sigma. 1-*O*-Methyl-STZ and 3-*O*-methyl-STZ were synthesized by Dr K. Tsujihara (Research Laboratories, Tanabe Seiyaku Co., Toda-shi, Japan). Medium 199 was obtained from Nissui (Tokyo). Fetal bovine serum (FBS) was purchased from Flow Laboratories (Sydney). Hypoxanthine, xanthine, *N*-methyl-*N*-nitrosourea (MNU), and an insulin assay kit (Insulin B-Test) were purchased from Wako (Osaka). 5,5-Dimethyl-1-pyrroline-*N*-oxide (DMPO) was obtained from Aldrich. Other chemicals used were of analytical grade.

Unless otherwise stated, ESR spectroscopy was performed as follows: 100 μl of 1 mM hypoxanthine or xanthine in Krebs-Ringer phosphate buffer (KRPB), 10 μl of 12.5 mU XOD and various concentrations of test chemicals were mixed and shaken vigorously for 30 s. Then 10 μl of DMPO was added, the mixture was again shaken for 5 s and the ESR spectrum was recorded promptly at room temperature (22°C) in a JEOL-FEIXG spectrometer (power 8 mW, field range 3320–3420 G, amplitude 1000, modulation 100 kHz, 2 G). In some experiments, 10 μl volumes of homogenates equivalent to 10^5 β-cells or 1.6 mg wet wt of hepatic tissue were examined in the same way in a total volume of 120 μl.

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Pancreatic β-cells were isolated from newborn Wistar rats by a reported method [9] and cultured in medium 199 containing 10% FBS, 10^2 U/ml penicillin G and 0.1 mg/ml of streptomycin sulfate. Reported methods were used for measurements of the activities of XOD [10] and SOD [11,12] in β-cells.

3. RESULTS AND DISCUSSION

The chemical structures of STZ and related compounds used in this work and their diabetogenic activities are shown in fig.1.

No ESR signal due to the DMPO-O_2^- radical adduct was observed on addition of STZ to a homogenate of pancreatic β-cells or liver in the presence or absence of hypoxanthine. However, when hypoxanthine was added, an ESR signal due to the DMPO-OH radical adduct was detected in both homogenates as exemplified in fig.2. This radical was thought to be a decomposition product of the O_2^- radical [13], because its signal disappeared on addition of SOD (16.4 U/ml). These results suggested that the homogenate of β-cells contained XOD activity. As far as we know, there is no report of XOD activity in β-cells, though high XOD activity has been found in whole pancreas [10]. Thus, we measured the XOD activity in cultured β-cells and estimated it to be 626 ± 2 mU/g cells (n = 5). This value was similar to that reported previously for whole pancreatic tissue.

The signal height of the ·OH radical increased with time in a homogenate of β-cells and 1.5 mM STZ enhanced the rate of its increase. In contrast, the signal of the ·OH radical in a liver homogenate decreased with time (fig.3).

In the hypoxanthine-XOD system, the signal of the O_2^- radical decreased rapidly when a liver homogenate was added, probably due to the high SOD activity in the latter, but in contrast, it did not change on addition of a homogenate of β-cells (not shown), suggesting that pancreatic β cells had very low SOD activity. In fact we later found that the SOD activity in β-cells was 472 ± 62 U/g cells (n = 5), which was only a small percentage of that in a liver homogenate [14].

As described, STZ enhanced the generation of O_2^- radicals in the hypoxanthine-XOD system with a homogenate of β-cells. Therefore, we next examined whether it had a similar effect in a cell-free system. A solution of STZ gave four signals due to DMPO-OH, but these were less than those of a solution of KRPB (not shown), indicating that

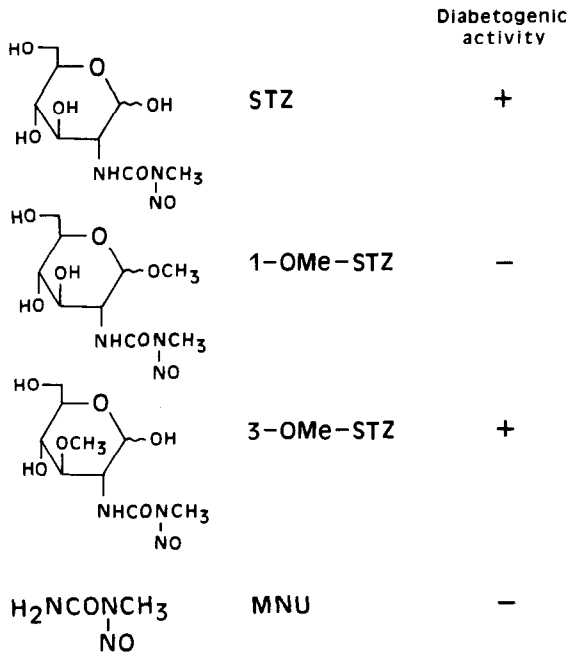

Fig.1. Chemical structures of STZ and related compounds. STZ, streptozotocin; 1-OMe-STZ, 1-*O*-methyl-streptozotocin; 3-OMe-STZ, 3-*O*-methyl-streptozotocin; MNU, *N*-methyl-*N*-nitrosourea.

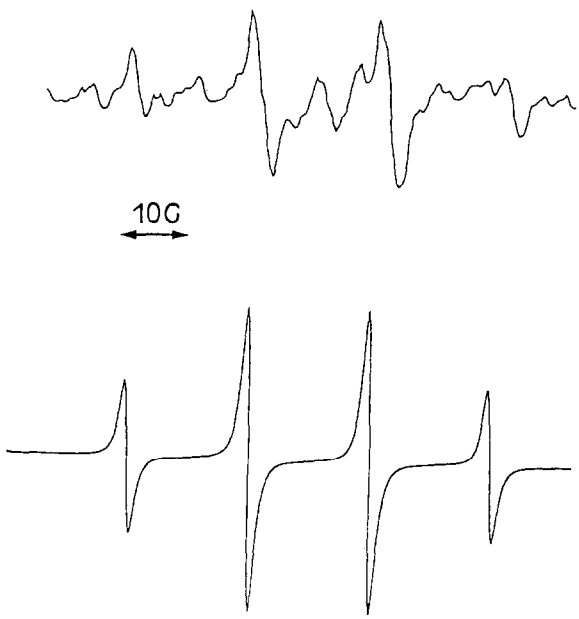

Fig.2. ESR spectrum of the DMPO-OH radical adduct obtained with a mixture of hypoxanthine and a homogenate of pancreatic β-cells. (Top) Observed spectrum; (bottom) computer-simulated spectrum.

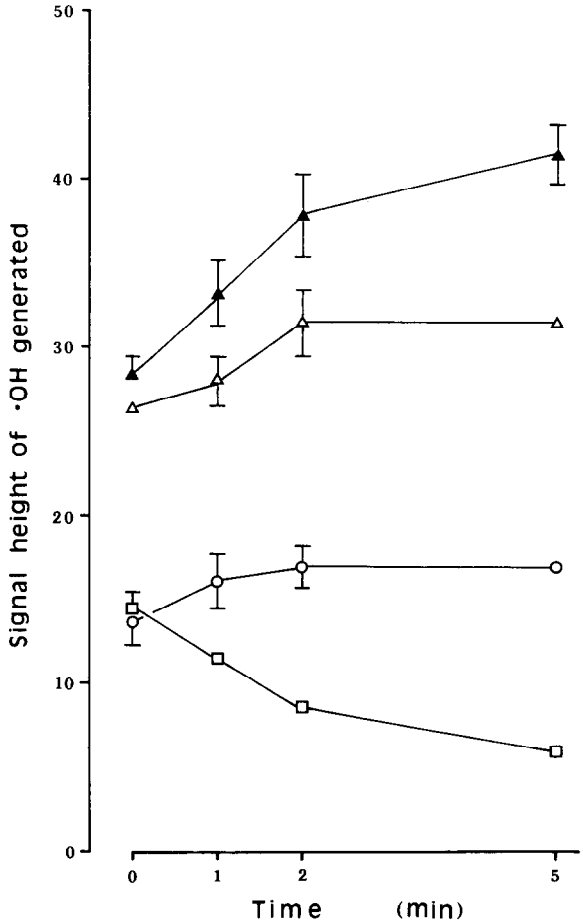

Fig.3. Time course of generation of DMPO-OH radical adducts in the hypoxanthine-XOD system. Addition: (○) none; (△) β-cell homogenate; (▲) β-cell homogenate plus STZ (1.5 mM); (□) liver homogenate. The ordinate indicates the signal height in arbitrary units of DMPO-OH at the lowest field. Values are means ± SE, or means only when the SE is less than 5% of the mean.

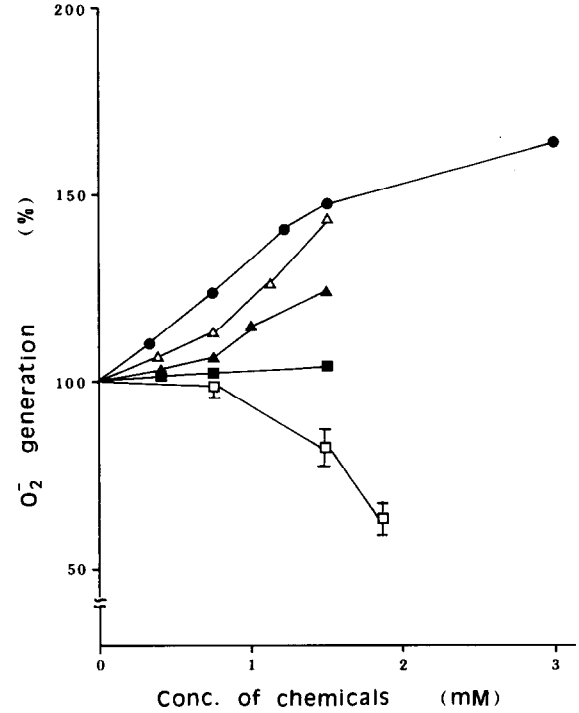

Fig.4. Effect of STZ and related compounds on generation of a DMPO-O_2^- radical adduct in the hypoxanthine-XOD system. Added compound: (●) STZ; (▲) 1-OMe-STZ; (△) 3-OMe-STZ, (■) MNU; (□) glucose. The ordinate indicates the amount of O_2^- generated as a percentage of that in the absence of test compounds. Values are means ± SE, or means only when the SE is less than 5% of the mean.

STZ itself had no radical generating activity. On addition of STZ to the hypoxanthine-XOD system, the intensity of the signal due to the O_2^- radical increased concentration-dependently to a maximum (150%) with 1.5 mM STZ. The diabetogenic STZ-derivative 3-OMe-STZ [15] had almost the same activity as STZ in increasing O_2^- radical generation, but the non-diabetogenic derivative 1-OMe-STZ examined in detail in our previous paper [15] had about half as much activity. MNU, a component of the STZ molecule, had no enhancing activity and glucose, another moiety of STZ, quenched O_2^- radical formation when added at high concentration (fig.4). Therefore, the molecular conformation of STZ seemed necessary for enhancement of the O_2^- radical generation. Similar results were obtained with xanthine instead of hypoxanthine as substrate (not shown). Computer simulation analysis indicated that the ESR signals consisted of those of O_2^- and OH radicals and that the ratio of intensities of the signals of these radicals was not affected by the chemicals added (not shown).

The present data showed that STZ and some of its analogues enhanced O_2^- radical generation in the XOD system, probably by a mechanism involving their interaction with XOD molecules. The molecular structure of a sugar moiety of STZ seems to be important for their interaction. The results also suggest that the cytotoxic effect of STZ on pancreatic β-cells may be related to its effect in enhancing generation of free radicals.

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