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Protection against Streptozotocin-induced Diabetes by Superoxide Dismutase

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Summary. Superoxide dismutase was administered intravenously to rats 50 min prior to intravenous administration of a diabetogenic dose of streptozotocin. A dose of 45 mg/kg streptozotocin alone produced marked glucose intolerance and a decrease in pancreatic insulin content to less than 10% of control; both of these effects were abolished by prior administration of 105 u/g of superoxide dismutase. Superoxide dismutase (105 u/g) administered 50 min before 65 mg/kg intravenous streptozotocin did not prevent the development of diabetes. The fall in pancreatic insulin content seen with streptozotocin alone was, however, partially reversed by superoxide dismutase.

Key words: Streptozotocin, diabetes, redox, superoxide dismutase protection.

Streptozotocin is a diabetogenic agent which causes beta cell necrosis and a permanent diabetes mellitus like syndrome [1, 2]. Chemically, it consists of a 1-methyl-1-nitrosourea linked to the 2 carbon of d-glucose. While its precise mode of action is unknown, it appears to alter the redox state of the affected cells. Specifically, streptozotocin has been shown to decrease pyridine nucleotide levels in isolated islets [3, 4], to decrease levels of reduced glutathione in erythrocytes [5], and to deplete erythrocytic and retinal levels of superoxide dismutase (Superoxide oxidoreductase erythrocuprin EC. 1:15.1.1 bovine) [6]. Superoxide dismutase (SOD) is a free radical scavenger which has been proposed as a major defence against the oxidizing effect of the superoxide radical [7]. This experiment was performed in order to determine if superoxide dismutase would protect against the diabetogenic effect of streptozotocin. If streptozotocin action is dependant in part on oxidation stress, with free radical production in excess of that which could be countered by normal cell mechanisms, then agents such as superoxide dismutase should provide some protection against its action.

Materials and Methods

Male Wistar rats weighing 200–250 g were used. All animals were fasted overnight prior to the administration of the pretreatment and treatment agents. Superoxide dismutase (Miles Research Laboratories) was given at a dose of 105 u/g body weight in 0.154 mol/l saline. The streptozotocin, (lot 1613E, U9889: Upjohn) was the generous gift of Dr. W. Dulin and was given in citrate buffer (0.1 mol/l, ph 4.5) at a dosage of 45 mg/kg or 65 mg/kg. All injections were given via the tail vein under light ether anaesthesia. Rats were injected with either SOD or 0.154 mol/l saline (control) fifty minutes prior to the administration of streptozotocin or saline. No rat received more than 0.4 ml total volume for the two injections. In a separate series SOD was given at the same time as the streptozotocin. In this experiment SOD and streptozotocin were prepared separately then drawn up into a single syringe immediately prior to injection. Five days after the appropriate injection schedule, glucose tolerance tests were carried out following an overnight fast. The animals were bled by cutting the tip off the tail and then collecting the freely flowing blood. After a baseline (0 min) sample the animal was given an IP glucose bolus (1 g/kg body wt.). Blood samples were subsequently taken at 15, 30 and 60 min. During all bleedings the animals were maintained under light ether anaesthesia. Blood samples were analysed for glucose using a Beckman glucose analyser. The animals were sacrificed under pentobarbital sodium, 0.5 ml, anaesthesia one hour after the glucose tolerance tests. The pancreases were removed and assayed for acid ethanol extractable insulin according to the technique of Scott and Fisher [8].

Results

I) Glucose Tolerance Tests

The results of the glucose tolerance tests are presented in Figure 1. The glucose tolerance of the animals treated with SOD plus saline and those animals

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receiving SOD 50 min prior to streptozotocin administration did not differ significantly from the saline plus saline-treated control animals. Those animals receiving streptozotocin after a control pretreatment with saline were profoundly diabetic, with serum glucose levels significantly higher than those in the saline plus saline controls ($p < 0.005$ at 0 & 15 min: $p < 0.0005$ at 30; and $p < 0.025$ at 60 min). In contrast those animals pretreated with SOD and 45 mg/kg streptozotocin showed consistently lower glucose values than those solely treated with streptozotocin, 45 mg/kg. The confidence levels of these differences were $p < 0.05$ at 0 min and $p < 0.005$ at 15, 30 and 60 min. However, administration of SOD simultaneously with streptozotocin had no effect on glucose tolerance. Similarly administration of SOD prior to streptozotocin 65 mg/kg did not produce statistically significant protection as reflected by glucose tolerance.

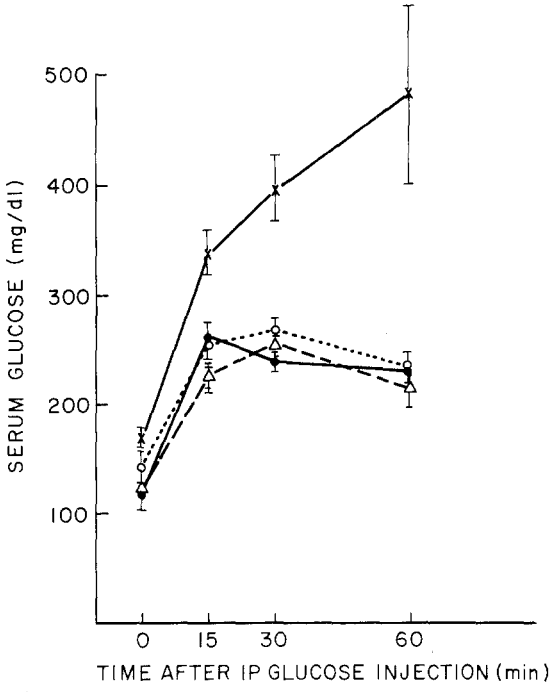

Fig. 1. Effect of superoxide dismutase (SOD) and streptozotocin on Glucose tolerance of rats. Serum glucose concentrations (vertical axis) plotted against time after intraperitoneal (IP) injection of glucose 1 g/kg body weight. Each point represents the mean and the bars the standard errors. The unbroken line with crosses represents glucose tolerance in animals treated with streptozotocin 45 mg/kg; the dotted line and open circles represents data from animals pretreated with SOD prior to streptozotocin 45 mg/kg. The dashed line with triangles represents data from animals treated with SOD and no streptozotocin and the continuous line with closed circles data from animals given neither SOD or streptozotocin

II) Extractable Insulin

It is evident that administration of both 45 mg/kg and 65 mg/kg streptozotocin markedly reduced islet insulin content (to < 10% of control) whereas administration of SOD alone has no effect (Table 1). Administration of SOD prior to 45 mg/kg streptozotocin prevented the streptozotocin-induced fall in islet insulin content. Streptozotocin 65 mg/kg markedly reduced islet insulin content to less than 1% of control values, this effect was partially reversed by prior injection of SOD ($p < 0.0005$), Table 1. Insulin content of pancreases of rats treated with simultaneous injection of streptozotocin and superoxide dismutase did not differ from those treated with streptozotocin alone.

Discussion

Pretreatment of rats with 105 u/g – animal-weight superoxide dismutase IV 50 min prior to administration of 45 mg/kg streptozotocin attenuated the diabetogenic effect of streptozotocin. This effect did not appear to be secondary to direct inactivation of streptozotocin by SOD, as injection of a mixture of

Table 1. Effect of superoxide dismutase on the pancreatic insulin content of rats injected with streptozotocin

Exp.	Injections		Pancreatic insulin content	*n*	Significance
	First	Second	μυ/g ($\chi \pm$ SEM)		
1.	Saline	Saline	495,195±61,325	6	
2.	Saline	Streptozotocin 45 mg/kg	47,584± 8,909	6	$v1$ <0.005
3.	SOD	Saline	530,822±31,569	6	$v1$ n. s.
4.	SOD	Streptozotocin 45 mg/kg	486,426±43,825	6	$v1$ n. s.
5.	Saline	Streptozotocin 65 mg/kg	13,500± 1,099	5	$v1$ <0.0005
6.	SOD	Streptozotocin 65 mg/kg	37,497± 3,180	6	$v5$ <0.005
7.[a]	SOD	Streptozotocin 45 mg/kg	50,369± 5,760	6	$v2$ n. s.

All agents were given intravenously. In all experiments the first injection preceded the second injection by 50 min with the exception of experiment 7. SOD refers to 105 u/g superoxide dismutase. [a]In this experiment SOD was administered immediately prior to the streptozotocin

the two agents did not provide protection. The effectiveness of SOD in this study contrasted with the previously reported ineffectiveness of a single dose in ameliorating diabetes induced by multiple doses of streptozotocin [9]. The different results may be explained by the known pharmacokinetics of the two drugs. Streptozotocin probably produces irreversable B cell damage between 10 & 30 min after IV administration as nicotinamide administered before or up to 10 min after streptozotocin injection [12] can prevent streptozotocin-induced diabetes but nicotinamide given 30 min after streptozotocin injection cannot. SOD given IV reaches a maximum concentration in marrow stem cells at about one hour, being present in such cells from 30 min to 30 h [13]. Thus, in contrast with the previous report the experimental design used in this study is such that maximum intracellular SOD concentrations could be anticipated at the time of action of the streptozotocin.

The fact that SOD, a free radical scavenger, can provide some amelioration of the diabetogenic effects of streptozotocin raises the possibility that the diabetogenic effect of streptozotocin may be mediated in part by streptozotocin-induced impairment of the ability of the B cell to withstand oxidant stress. The mechanism through which streptozotocin may mediate this effect on cell redox metabolism is unknown. However, such streptozotocin-induced redox impairment could result from: First, an increase in the production of intracellular free radicals, either endogenous or exogenous (e. g. streptozotocin itself), or second from a streptozotocin-induced decrease in the ability of the cell to maintain antioxidant mechanisms (e. g. inability to maintain reduced glutathione concentrations or a reduction in superoxide dismutase levels). In favour of the former possibility are the observations that nicotinamide can protect against the diabetogenic and haemolytic effects of streptozotocin [10–12, 14] and that streptozotocin reduces red cell [5] and islet (unpublished) GSH concentration. In favour of the latter is the observed effect of streptozotocin in decreasing superoxide dismutase activity [6]. Thus, while it is evident that the precise mechanism through which streptozotocin may influence redox metabolism in B cells is not clear there is a body of data supporting such a general role [15].

Investigation of the antitumour effects of streptozotocin have led to alternative hypotheses regarding its mode of action. For example, it has been demonstrated that streptozotocin can produce DNA damage [16] and suggested that repair of same by poly (ADP-ribose) polymerase, which utilises NAD [17] results in the observed reduction of cellular NAD [3–5] and that the protective effect of nicotinamide could be exerted by preventing this fall in NAD. Such a mechanism could explain the ability of nicotinamide to protect against a fall in reduced glutathione in that reduced nicotine adenine dinucleotide is required for reduced glutathione production. Further, the fall in reduced glutathione could result in a greater production of free radicals and greater reliance on free radical scavengers such as superoxide dismutase. While the above hypotheses are both attractive it is necessary to recall that not only are there species differences in the susceptibility to the effect of streptozotocin but that there may also be differences between tissues with respect to the mechanism of action. For example; the effect of streptozotocin on guanylate cyclase differs from cell type to cell type [18] and the antitumour and diabetogenic actions of streptozotocin can be dissociated [10]. These facts indicate the need for caution in extrapolating from one system to another.

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