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Effect of l-Methyl-l-Nitrosourea and Streptozotocin on Glucose-Induced Insulin Secretion by Isolated Islets of Langerhans*

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Summary. The present experiments were designed to compare the effects of streptozotocin and l-methyl-l-nitrosourea upon glucose-induced insulin secretion by isolated islets of Langerhans. Both drugs depressed the insulin response at one and two hours incubation but higher molar concentrations of the nitrosourea were required to produce the same level of inhibition as streptozotocin, a difference perhaps related to the latter's glucose moiety.

Key words: l-methyl-l-nitrosourea, streptozotocin, insulin secretion.

Streptozotocin (ST), an antibiotic, antitumor and diabetogenic agent composed of glucose and l-methyl-l-nitrosourea (MNU), [1] produces irreversible hyperglycemia in rats, dogs, mice and rhesus monkeys [2, 3, 4]. The permanent diabetogenic property of ST can be explained by its B-cell cytotoxic action [5] and its early hyperglycemic action is thought to be due to a lowering of tissue nicotinamide adenine dinucleotide (NAD) content, both actions being blocked by nicotinamide [6–15]. Earlier, we found that ST in vitro causes a dose-related suppression of glucose-induced insulin secretion by isolated islets of Langerhans, an effect which is also blocked by nicotinamide [16].

MNU similarly lowers brain, liver and pancreatic NAD content and like ST inhibits islet tissue action potentials in vitro [17–20]. MNU has recently been shown to cause hyperglycemia in the Chinese Hamster [21].

The purpose of the present study was to compare the effect of MNU and ST on glucose-induced insulin secretion by isolated islets of Langerhans.

Materials and Methods

Islets were isolated from fed, male albino rats (300–350 gm) by the method of Lacy and Kostianovsky [22]. Groups of eight islets were transferred to micro-incubation flasks and incubated in a bicarbonate-buffered medium (1.0 ml) containing albumin (5 mg/ml) and equilibrated with oxygen (95 per cent) and carbon dioxide (5 per cent), according to a method described previously [23]. Control media contained glucose alone (3 mg/ml). Test media contained glucose (3 mg/ml) together with either ST (Upjohn lot #9164-VDV-136)* or MNU (National Cancer Institute). The pH of test media was adjusted to be equal to that of the control media (pH 7.4). The flasks were then incubated for two successive one-hour incubations at 36° C in a Dubnoff metabolic shaker. After the first hour the media were removed and only control media added for the second-hour incubation. The insulin content of the media was assayed according to the immunoassay of Wright et al. [24], as applied to isolated islets.

In the control media containing glucose alone (3 mg/ml) the mean absolute rate of insulin release averaged 182 ± 10 and 187 ± 10 μU/islet per hour ($n_1 = 23$; $n_2 = 23$), respectively, for the first and second hour of incubation. In the table of results, the rates of insulin secretion found in the test media are expressed in per cent of the mean control value (glucose alone) found within the same experiment and during the same period of incubation.

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Table 1. Effect of streptozotocin (ST) and varying concentrations of l-methyl-l-nitrosourea (MNU) on insulin secretion (mean ± SEM expressed as per cent of mean control rate found within the same experiment, with statistical significance of each compared with its own mean control)

	First hour						Second hour				
Drug	Dose mg/ml	Dose mM	Insulin output Mean ± SEM	Replicates	p	Drug	Dose mg/ml	Dose mM	Insulin output Mean ± SEM	Replicates	p
ST	0.5	1.88	56.7 ± 2.4	18	< 0.001	Nil	–	–	18.8 ± 3.0	18	< 0.001
MNU	0.194	1.87	114.0 ± 11.7	5	> 0.10	Nil	–	–	97.0 ± 9.2	4	> 0.25
MNU	0.23	2.23	101.9 ± 8.7	4	> 0.25	Nil	–	–	91.6 ± 6.9	5	> 0.15
MNU	0.28	2.72	53.5 ± 3.3[a]	7	< 0.001	Nil	–	–	23.0 ± 1.9[b]	7	< 0.001
MNU	0.5	4.85	51.6 ± 7.6[a]	11	< 0.001	Nil	–	–	18.2 ± 3.9[b]	11	< 0.001

[a] compared with line 1, ST (0.5), $p > 0.20$; $p > 0.20$
[b] compared with line 1, ST (0.5), $p > 0.20$; $p > 0.25$

Results

The results are summarized in Table 1. In the first line, ST present only during the first hour at a concentration of 0.5 mg/ml (1.88 mM) depressed insulin secretion to 56.7 ± 2.4 per cent of control over the first hour and to 18.8 ± 3.0 per cent of control over the second hour-results which are similar to those obtained previously [16]. The next four lines represent the effect of increasing concentrations of MNU and demonstrate no significant inhibition at 0.194 mg/ml (1.87 mM) and 0.23 mg/ml (2.23 mM) over either hour, but significant inhibition of insulin secretion at 0.28 mg/ml (2.72 mM) and 0.5 mg/ml (4.85 mM) over both hours. The level of inhibition at these latter concentrations of MNU is, in fact, statistically the same as that obtained by ST at 0.5 mg/ml.

Discussion

The present results demonstrate a dose-related depression of glucose-induced insulin secretion in vitro by MNU similar to that previously seen with ST [16]. It is of importance that when equimolar amounts of ST and MNU were tested, only ST depressed insulin secretion. On a mole for mole basis more MNU was required to produce the same effect as ST. St's enhanced potency could be due to its glucose subunit acting as a carrier of the active component, MNU, into the B-cell. The latter theory was initially invoked to explain the inability of MNU alone to produce diabetes in certain species [7]. What we may be seeing in vitro using insulin secretion as an index may be the effect of delivery of higher molar concentrations of MNU directly to the target organ when the latter is combined with glucose as ST.

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