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Hyperglycemic Activity of Some Non-Nitrosated Streptozotocin Analogs (39359)

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Streptozotocin, an antitumor and diabetogenic agent derived from glucose and 1-methyl-1-nitrosourea (1) has been shown to produce irreversible hyperglycemia in rats, dogs, mice, and rhesus monkeys (2–4). Diabetes onset after intravenous streptozotocin follows a triphasic pattern with an early hyperglycemia (peaking at the second hour), followed by hypoglycemia (most marked at the seventh hour), and finally, by permanent hyperglycemia by the twenty-fourth hour.

The detailed mechanism for streptozotocin-induced blood glucose changes is not completely understood. The work of Junod *et al.* (5) assigns the permanent diabetogenic property of streptozotocin to its β-cell cytotoxic action. The early hyperglycemic phase is believed to result from inhibition of β-cell insulin secretion (6). Since streptozotocin decomposes rapidly at physiological pH to liberate diazomethane, a classic alkylating agent, various authors (6–8) have suggested that streptozotocin and related *N*-nitroso compounds exert their actions via release of diazoalkane. This possibility has been explored in the following work by evaluating the effects of non-nitrosated as well as nitrosated analogs of streptozotocin on rat blood glucose.

Materials and methods. Streptozotocin was kindly supplied by Dr. G. C. Gerritsen, the Upjohn Company, Kalamazoo, Michigan. The syntheses of (I)–(IV), the haloethyl streptozotocin analogs, and of (V)–(VIII), the *O*-acetylated *N*-aryl streptozotocin analogs, have been reported elsewhere (9).

Male Sprague–Dawley rats (Charles River Laboratories) of 210–250 g body weight were used throughout. Prior to use, all animals were kept at 30–31° and fed standard Wayne chows *ad libitum*. For measurement of effects on fasting blood glucose, rats previously fasted 48 hr were bled from the tail vein before dosing and 0.5, 1, 2, 3, 4, 5, 6, and 48 hr after intraperitoneal administration of drug or vehicle. After the sixth hour bleeding, all animals were fed rat chow *ad libitum*. Twenty-four and forty-eight hours after dosing, all animals were tested for glycosuria using Lilly Tes-Tape. Complete details of the methods for determining blood glucose and for rat oral glucose tolerance testing have been described elsewhere (10). After completion of the glucose tolerance tests, all animals were provided with food *ad libitum* and were tested for glycosuria 24–48 hr after administration of drug or vehicle. For the fasting blood glucose experiments and the glucose tolerance tests using streptozotocin and compounds I–IV, the compounds were administered within 5 min after dissolution in 0.01 *M* citrate buffer, pH 4.5. The non-nitrosated analogs (V–VIII) were given during glucose tolerance tests as a suspension in 0.5% methylcellulose.

Results. The effect of streptozotocin and the streptozotocin analogs on rat glucose tolerance is shown in Table I. Streptozotocin and all eight analogs in doses of 100 mg/kg ip produced significant increases in blood glucose compared to vehicle-treated animals. However, none of the analogs produced a sustained rise in blood glucose comparable to the parent compound. The non-nitrosated analogs (II) and (IV) produced greater decreases in glucose tolerance in doses of 100 mg/kg ip than their nitrosated counterparts (Analogs I and III). Only streptozotocin treated animals exhibited glycosuria 24–48 hr after dosing.

Several of the compounds were also studied for their ability to affect fasted rat blood glucose levels. The results are shown in Table II. Streptozotocin (100 mg/kg, ip) and

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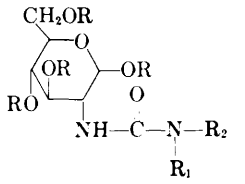
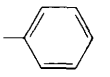
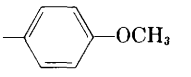
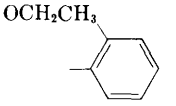
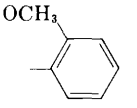


	R	R_1	R_2
Streptozotocin	H	—N=O	$-CH_3$
Analog (I)	H	—N=O	$-CH_2CH_2Cl$
(II)	H	—H	$-CH_2CH_2Cl$
(III)	$-\overset{\overset{O}{\|}}{C}-CH_3$	—N=O	$-CH_2CH_2Cl$
(IV)	$-\overset{\overset{O}{\|}}{C}-CH_3$	—H	$-CH_2CH_2Cl$
(V)	$-\overset{\overset{O}{\|}}{C}-CH_3$	—H	
(VI)	$-\overset{\overset{O}{\|}}{C}-CH_3$	—H	
(VII)	$-\overset{\overset{O}{\|}}{C}-CH_3$	—H	
(VIII)	$-\overset{\overset{O}{\|}}{C}-CH_3$	—H	

the nitrosated analog (I) (800 mg/kg, ip) produced the early hyperglycemic phase followed by a rapid lowering of blood glucose. All animals treated with (I) died between 7–24 hr after treatment presumably as the result of severe hypoglycemia. Streptozotocin also has been reported (11, 12) to produce lethalities 10 hr after administration of doses which produced a hypoglycemia effect comparable to that observed in the present studies with (I). In the present studies, the animals treated with 100 mg/kg ip of streptozotocin showed only modest hypoglycemia but had glycosuria 24–48 hr after dosing. The non-nitrosated analogs (II), (IV), (V), and (VII) also produced early hyperglycemia; however, neither the hypoglycemic phase nor permanent hyperglycemia and glycosuria were observed.

Discussion. Golden and co-workers (6) have demonstrated recently that streptozotocin can exert a direct inhibitory effect on glucose-induced insulin secretion by isolated islets of Langerhans. This indicates that a direct action on the β-cell, rather than a secondary liberation of glycogenolytic agents, such as epinephrine, is responsible for the early hyperglycemic phase after streptozotocin treatment. Since streptozotocin under various conditions has been found to be unstable, these authors postulated that the breakdown product diazoalkane was responsible for the early hyperglycemic phase. However, the present studies using streptozotocin analogs which cannot form diazoalkane breakdown products do not substantiate their conclusion. Not only did the nitroso analogs (I), (III), and streptozotocin produce early hyperglycemia but so did the non-nitrosated streptozotocin analogs. Moreover, the data suggest that the sugar moiety may be acting as more than just a nonspecific hydrophilic carrier for nitrosourea groups as has been suggested by numerous investigators (13–15) but may actually have a more direct effect.

Even though the non-nitrosated analog (II) produced more severe glucose intoler-

TABLE I. EFFECT OF STREPTOZOTOCIN ANALOGS ON RAT ORAL GLUCOSE TOLERANCE.[a]

Expt. no.	Compound	N[b]	Blood glucose (mg/100 ml) at minutes after glucose $\bar{X}$ ± SEM						
			0	30	60	90	120	150	180
1	Vehicle	4	56 ± 1	105 ± 1	89 ± 1	83 ± 1	71 ± 1	66 ± 2	60 ± 2
	Streptozotocin	3	74 ± 3***	149 ± 4***	201 ± 5***	225 ± 7***	215 ± 4***	204 ± 3***	202 ± 9***
	(IV)	3	69 ± 4*	129 ± 1***	–	105 ± 2***	–	101 ± 1***	–
2	Vehicle	4	47 ± 2	135 ± 3	111 ± 5	101 ± 2	91 ± 4	95 ± 3	86 ± 3
	(I)	3	96 ± 5***	120 ± 5	123 ± 6	142 ± 5***	139 ± 6***	109 ± 2**	110 ± 3***
	(II)	3	89 ± 5***	208 ± 9***	213 ± 6***	178 ± 9***	140 ± 5***	123 ± 6**	117 ± 8**
3	Vehicle	4	44 ± 1	128 ± 2	113 ± 4	96 ± 3	90 ± 3	91 ± 2	–
	(III)	3	67 ± 2	128 ± 3	131 ± 2**	158 ± 4***	131 ± 4***	128 ± 5***	–
	(IV)	3	78 ± 3***	180 ± 5***	205 ± 4***	172 ± 3***	134 ± 6***	117 ± 2***	–
4	Vehicle	6	79 ± 4	170 ± 6	144 ± 4	121 ± 3	106 ± 4	95 ± 2	–
	(V)	3	86 ± 8	269 ± 8**	187 ± 10**	135 ± 4	118 ± 7	106 ± 7	–
	(VI)	3	89 ± 8	180 ± 16	189 ± 13**	154 ± 13*	124 ± 12	111 ± 5*	–
	(VII)	3	106 ± 5**	219 ± 12*	210 ± 7***	153 ± 5***	122 ± 2***	109 ± 3**	–
5	Vehicle	3	68 ± 2	172 ± 7	119 ± 7	90 ± 2	82 ± 2	78 ± 1	68 ± 2
	(VIII)	3	82 ± 3*	150 ± 9	164 ± 5*	128 ± 5*	100 ± 7	86 ± 2*	71 ± 2

[a] Compounds, 100 mg/kg, ip, or vehicle were given 30 min prior to oral administration of 1 g of glucose per kg body weight.
[b] Number of rats studied.

TABLE II. EFFECT OF STREPTOZOTOCIN AND STREPTOZOTOCIN ANALOGS ON 48-HR FASTED RAT BLOOD GLUCOSE.[a]

Treatment	Dose (mg/kg, ip)	Blood glucose (mg/100 ml) at minutes after glucose $\bar{X}$ ± SEM								
		Initial	0.5	1	2	3	4	5	6	48
Vehicle	–	75 ± 6	81 ± 4	83 ± 3	69 ± 5	80 ± 4	87 ± 4	89 ± 3	88 ± 3	69 ± 3
(II)	200	69 ± 3	164 ± 10***	136 ± 7***	135 ± 5***	136 ± 6***	127 ± 7**	139 ± 8**	115 ± 4**	69 ± 6
	100	72 ± 3	136 ± 7***	137 ± 2***	108 ± 2***	112 ± 6**	105 ± 3**	101 ± 11	102 ± 8	86 ± 4*
(IV)	200	67 ± 1	122 ± 4***	132 ± 5***	131 ± 7***	136 ± 6***	143 ± 5***	123 ± 2***	113 ± 1***	81 ± 2*
	100	69 ± 1	125 ± 1***	135 ± 3***	139 ± 6***	114 ± 1***	102 ± 2**	105 ± 1**	121 ± 4***	88 ± 4*
Streptozotcin	100	79 ± 3	127 ± 2***	151 ± 3***	210 ± 2***	270 ± 4**	318 ± 9***	204 ± 7***	82 ± 5	484 ± 2***
Vehicle	–	67 ± 2	69 ± 1	77 ± 2	67 ± 2	65 ± 2	67 ± 2	66 ± 2	71 ± 4	123 ± 5
(I)	800	60 ± 2	94 ± 5**	114 ± 1***	156 ± 6***	79 ± 3*	48 ± 3**	25 ± 2**	23 ± 3***	–[b]
(II)	800	62 ± 1	111 ± 2***	140 ± 3**	158 ± 4***	158 ± 5***	130 ± 1***	128 ± 3***	118 ± 3***	129 ± 9
(V)	400	61 ± 2	80 ± 1**	87 ± 3**	85 ± 4*	80 ± 3*	84 ± 3**	86 ± 6*	101 ± 2***	111 ± 7
(VII)	365	62 ± 1	80 ± 5	85 ± 2***	88 ± 5**	102 ± 2***	97 ± 4***	92 ± 7**	81 ± 2*	118 ± 2

[a] Four rats per group. Significance compared to controls determined by Student's t test, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.
[b] Lethal, 4/4.

ance at 100 mg/kg ip (Table I) and at least as great a fasting hyperglycemia in doses of 800 mg/kg ip (Table II) as its corresponding nitrosated analog (I), only (I) induced a rapid phase of blood glucose lowering similar to streptozotocin (5) 5 to 6 hr after dosing with subsequent lethality of all animals. This finding suggests that the events that occur during the early hyperglycemic phase may be unrelated to the subsequent hypoglycemic and permanent diabetic phases and further emphasizes the importance of the nitroso group in the β-cell cytotoxic action of streptozotocin.

Analog (III) which is referred to as GCNU or NSC 114460, is one of the most promising antitumor agents of the *N*-alkyl nitrosourea family (16) and was previously reported by Schein and coworkers to lack hyperglycemic activity (17). They found that rat blood glucose levels measured 48 hr after the administration of 200 mg/kg of (III) were within the normal range. However, our data with GCNU and its nonacetylated analog (I), known as DCNU or chlorozotocin (18), have shown that transient hyperglycemia can be produced in doses of 100 mg/kg ip in rat glucose tolerance tests. This finding plus the similarity of carbohydrate perturbations suggest that caution should be exercised when these agents are used in the clinical treatment of tumors.

Finally, although the non-nitrosated analogs of streptozotocin, (II) and (IV)–(VIII), have been proven to be inactive against L1210 lymphoid leukemia and against human epidermoid carcinoma of the nasopharynx (cell culture) (19), their pancreatotrophic effect without production of frank diabetes indicates potential utility as pancreatic radiopharmaceuticals. Radioiodinated analogs of several of these streptozotocin derivatives are presently under study with respect to their ability to image islet cell malignancies (20).

Summary. Six non-nitrosated and three nitrosated analogs of the antitumor and diabetogenic agent, streptozotocin, have been found to produce hyperglycemia in fasted rats. The hyperglycemia produced by the non-nitrosated analogs was of early onset and did not result in the production of permanent diabetes. The data emphasizes the important role of the nitroso group of streptozotocin in the production of the hypoglycemic and permanent diabetic phases while the sugar moiety may have a direct influence on production of the early hyperglycemia phase.

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