Biochemical Pharmacology, Vol. 30, No. 9, pp. 925–930, 1981.
Printed in Great Britain.

0006-2952/81/090925-06 $02.00/0

THE EFFECT OF STREPTOZOTOCIN DIABETES ON ENDOTOXICOSIS IN MICE*

M. K. AGARWAL†, J. PAILLARD‡, M. PHILIPPE¶ and D. BLONDEL‖
Centre National de la Recherche Scientifique, Paris, France

(*Received* 28 *August* 1980; *accepted* 27 *October* 1980)

Abstract—Repeated injections of streptozotocin (SZN), to a total of 5 mg, produced a state of diabetes in mice without alteration in various parameters of the reticuloendothelial system (RES). This was associated with hyperreactivity to endotoxin lethality which decreased with time and which could not be correlated with blood glucose levels in SZN injected animals. One single injection of SZN, too, sensitized animals to endotoxin death, albeit less effectively than after repeated administration of small doses, although the effect was dose dependent. Impairment of protection by cortisol against endotoxin lethality in mice was less severe after a single injection of SZN vs administration of an equivalent amount of SZN divided over a 5 day period. Blood glucose levels diminished very rapidly after endotoxin administration and were only partially reversed during cortisol protection against the lethal effects of endotoxin in SZN pretreated animals. Streptozotocin impaired the induction of tolerance to endotoxin but not the activation of the RES that accompanies tolerance. It is suggested that the influence of various pharmacological agents that reverse SZN diabetes may form a new model to elucidate the pathophysiology of endotoxicosis.

Hypoglycemia is one of the various host parameters said to be causal in the death of mice injected with lethal doses of bacterial endotoxins (for recent reviews see refs. 2 and 3). Hyperreactivity to endotoxin lethality has been correlated with the severity of blood sugar depletion [5] although neither alloxan diabetes [16] nor intermittent glucose administration by either the intravenous [8] or the oral [12] routes protects animals against death from endotoxin. Continuous glucose infusions, however, have been reported to afford protection against lethality in dogs [10].

Streptozotocin (SZN: 2-deoxy-2[3-methyl-3-nitrosoureido]-D-glucopyranose) is a broad spectrum antibiotic with a wide range of effects on the host [6, 7, 9, 11, 13–15, 17, 18]. The diabetic state produced by this agent is more severe and the biological manifestations more comparable to clinical diabetes than after alloxan [6, 7, 9, 11, 13–15, 17, 18]. It was of obvious interest therefore to assess the influence of SZN diabetes on the course of endotoxicosis. The results are embodied in this report.

MATERIALS AND METHODS

Male, Swiss mice (25 ± 2 g) were housed with free access to pellet food and water. Streptozotocin (courtesy Upjohn, Kalamazoo, Michigan), lot 1613 E, was dissolved in citrated saline, pH 4.5, immediately prior to intraperitoneal (i.p.) administration in 0.5 ml volumes containing 1 mg of the product for five successive days. In another series, one single i.p. injection of varying amounts of SZN was given with endotoxin challenge.

Cortisol (Sigma) or triamcinolone acetonide (Sigma) was suspended in saline and injected i.p. in 1 mg amounts in a volume of 0.5 ml. *S. typhimurium* W endotoxin (lot 654477) was purchased from Difco, dissolved in isotonic saline and injected in 0.5 ml volumes i.p. in all cases. The challenge dose of endotoxin is indicated at appropriate places in the paper; survivors after challenge were recorded 48 hr later.

Tolerance to endotoxin was established by daily i.p. injections of 5, 5, 10, 10 and 20 μg of the above preparation of endotoxin for five successive days. Animals so treated were used 48 hr after the last injection.

Blood glucose levels were determined on heparinized samples from retro-orbital plexus centrifuged to obtain plasma. 20 μg plasma samples were mixed with 2.5 ml Gluci-net reagent (Sclavo Diagnostics, Via Fiorentina 1, Siena, Italy) containing phosphate buffer pH 7.5, *A. niger* glucose oxidase, horseradish peroxidase, 4-aminophenazone and hydroxybenzoate. This colorimetric procedure is routinely used for clinical diagnosis and is based on the principle where D-glucose, in the presence of glucose oxidase, is transformed to hydrogen peroxide and D-gluconate which is then converted to a coloured chromogen in presence of peroxidase. The intensity of the stable pink colour is measured at 510 nM against a blank. A standard D-glucose test was run in each case. The results are expressed in either g/l or mg per cent.

The functional activity of the RES was assessed by the carbon clearance test [4] as previously adapted [1]. Briefly, 20 mg/100 g body weight of carbon (C11

* Supported by a grant from UER Broussais Hôtel-Dieu.
† Maître de Recherche au CNRS to whom all correspondence should be sent at 15 rue de l'Ecole de Médecine, 75270 Paris Cédex 06, France.
‡ Research Associate.
¶ INSERM Assistant.
‖ Doctoral student.

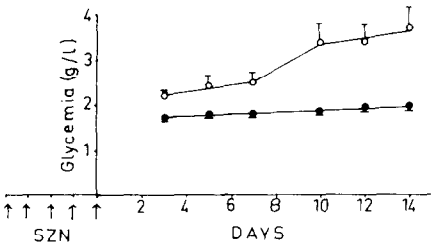

Fig. 1. Streptozotocin induced diabetic state in mice. Animals were given either the citrated saline (●) or 1 mg streptozotocin in this buffer (○), intraperitoneally, for five successive days. Blood sugar levels were determined at various times indicated in the figure, from the same group of animals. All results are expressed as g/l ± the standard error of the mean on 10 individual assays.

1431 a, Gunther Wagner, Hanover, Germany) in saline was administered i.v. in 0.2 ml. Blood samples of 50 μl from the retro-orbital plexus were withdrawn 5 and 10 min later and lysed in 4 ml of 0.1% Na_2CO_3 solution. The quantity of carbon was measured at 675 μm and compared against a standard curve.

The significance between mean responses following different treatments was determined by the *t* test. The Chi square test was used to assess significance between survival rates after challenge with endotoxin. A standard deviation was calculated for all mean values. The data in Fig. 1 are plotted ± the standard error.

RESULTS

Data in Figure 1 show that after five daily i.p. injections of 1 mg each of streptozotocin, glycemia exhibited a biphasic rise. A slower increase in blood sugar until day 7 post treatment was followed by a second burst between days 7 and 10 and the glycemia attained 4 times the control level on day 14. These confirm earlier results [6, 9]. Animals so treated exhibited no symptoms of toxicity and were used to assess endotoxin lethality.

Data in Table 1 show that endotoxin lethality in buffer pretreated animals was proportional to the amount of the challenge dose of the toxin and that 1 mg cortisol, given i.p. concurrently with endotoxin, afforded 100% protection against death. This sort of treatment with citrated physiological saline did not alter the response of mice to endotoxin as compared to normal, untreated animals (not shown). At each challenge dose of the toxin, more mice died in the SZN pretreated (5 × 1 mg i.p.) group than in the buffer injected controls. Furthermore, cortisol was less effective in protection of these SZN pretreated mice against endotoxin challenge. Since serum glucose levels were significantly elevated ($P < 0.001$) as a result of SZN pretreatment (Fig. 1), initial blood sugar level does not *per se* appear to reverse death from endotoxin. Data in Table 1 furthermore show that the SZN effect wears off with time. Sensitization to endotoxin lethality (500 μg) was maximum (20% survival) immediately after SZN pretreatment but the number of survivors increased to 53 per cent and 77 per cent two and three weeks, respectively, after the last injection of SZN. Since blood sugar levels attain a plateau within the first two weeks after pretreatment (Fig. 1), the explanation for near normal reactivity to endotoxin at the three week time period after SZN pretreatment should be sought in other host parameters.

Data in Table 2 show the effect of acute doses of SZN on endotoxin lethality in mice. Animals were sensitized by as little as 3mg of SZN administered concurrently with the endotoxin challenge and the effect was dose dependent. However, the total of 5 mg of SZN was more effective when administered

Table 1. Sensitization to the lethal effects of endotoxin in mice after chronic treatment with streptozotocin

Endotoxin	Living/ total	% Survival	Statistics
Buffer			
1. 100 γ	20/20	100	
2. 500 γ	15/20	75	2 vs 1: $P < 0.02$
3. 500 2 weeks	11/15	73	3 vs 1: $P < 0.01$
4. 500 3 weeks	18/20	90	4 vs 1: N.S.*
5. 1000 γ	12/60	20	5 vs 1: $P < 0.001$
6. 1000 + cortisol	20/20	100	6 vs 5: $P < 0.001$
Streptozotocin			
7. 100γ	16/20	80	7 vs 1: $P < 0.05$
8. 500 γ	4/20	20	8 vs 2: $P < 0.001$
9. 500 2 weeks	8/15	53	9 vs 8: $P < 0.05$
10. 500 3 weeks	13/17	77	10 vs 9: N.S.; vs 8: $P < 0.001$
11. 1000 γ	2/20	10	11 vs 5: N.S.
12. 1000 + cortisol	8/20	40	12 vs 11: $P < 0.05$ 12 vs 6: $P < 0.001$

* N.S. = Not significant. Cortisol = 1 mg i.p. with endotoxin challenge.

Citrated saline buffer or streptozotocin was given for five successive days i.p. in 0.5 ml volume to a total of 5 mg. Animals were challenged 48 hr, 2 weeks and 3 weeks later.

Table 2. Influence of an acute dose of streptozotocin on the lethal effect of endotoxin

Streptozotocin	Living/ total	% Survival	Statistics
1. None	47/60	78	
2. 1 mg	14/20	70	2 vs 1: N.S.*
3. 3 mg	11/20	55	3 vs 1: N.S.
4. 5 mg	8/20	40	4 vs 1: $P < 0.005$
5. 10 mg	2/20	10	5 vs 1: $P < 0.001$
6. 5 mg + cortisol	12/20	60	6 vs 4: N.S. 6 vs 1: N.S.

* N.S. = Not significant.

The indicated dose of streptozotocin was given with 500 γ endotoxin, i.p. in all cases. Cortisol was given in 1 mg amounts with endotoxin i.p.

over a 5 day period (Table 1) than as a single dose (Table 2) both in sensitizing to endotoxin lethality and in impairing protection by cortisol.

Data in Table 3 show that within 6 hr as little as 100 μg (a sublethal dose) of endotoxin lowered blood glucose levels in both the control and the SZN groups and these decreased slightly more 24 hr after endotoxin administration. Thus, as in studies by others after alloxan [16], it was not possible to maintain increased glycemia of the diabetic state once endotoxin had been administered. It is especially noteworthy that serum glucose levels were not significantly different after endotoxin injection in saline vs SZN group although the latter had significantly more ($P < 0.001$) blood sugar as a result of SZN treatment. The fate of carbohydrates after endotoxin injection has been speculated but is unknown [5, 16].

Data in Table 3 furthermore show that even triamcinolone acetonide (TA), a steroid with nearly 10-fold greater potency than cortisol, did not increase glycemia of endotoxin treated mice during the first 6 hr but blood glucose was significantly elevated 24 hr after the hormone in SZN pretreated group. The significant drop in blood glucose 24 hr after TA administration in control mice can best be explained as a feedback insulin release after initial hyperglycemia (6 hr time point) and that this regulatory mechanism was wanting in SZN treated group due to β-cell destruction. More important, glycemia was nearly identical after simultaneous administration of only 100 μg of endotoxin and TA (the potent synthetic steroid) in both the buffer and the SZN pretreated groups. This was significantly below the normal control level at all time points, and not significantly different from that seen after endotoxin injection in either group in the first 6 hr. However, combined administration of the hormone and the toxin did elevate glycemia above the group given endotoxin alone at the 24 hr time point but this was far less than the level after TA injection in SZN treated mice. Thus, hormone protection against endotoxin is not paralleled by restoration of glycemia.

Data in Table 4 show that five, daily i.p. injections

Table 3. Effect of endotoxicosis on blood sugar levels in Streptozotocin treated mice receiving a sublethal dose of endotoxin

Treatment*	Glycemia (mg/100 ml)			
	6 hr		24 hr	
	Saline	Streptozotocin	Saline	Streptozotocin
1. Saline	177 ± 21 (9)	228 ± 37 (10)	177 ± 21 (9)	228 ± 37 (10)
2. E	86 ± 9 (5)	93 ± 10 (5)	77 ± 15 (7)	79 ± 12 (5)
3. TA	189 ± 14 (5)	249 ± 30 (5)	141 ± 14 (6)	354 ± 91 (5)
4. E + TA	104 ± 27 (5)	111 ± 12 (5)	133 ± 10 (6)	138 ± 28 (6)
Statistics				
2 vs 1	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
3 vs 1	N.S.†	N.S.	$P < 0.005$	$P < 0.02$
4 vs 1	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
4 vs 2	N.S.	N.S.	$P < 0.001$	$P < 0.001$
4 vs 3	$P < 0.001$	$P < 0.001$	N. S.	$P < 0.001$

* E = Endotoxin 100 γ i.p. TA = Triamcinolone acetonide 1 mg i.p.

† N.S. = Not significant. Mean ± standard deviation; sample size in parentheses.

Animals were injected with either buffer or streptozotocin for five days prior to test as in Table 1 and used 48 hr after the last injection.

Table 4. Effect of streptozotocin pretreatment on the establishment of tolerance to endotoxin

Endotoxin	Living/ total	% Survival	Statistics
1. 0.5 mg	17/20	85	
2. 1.0 mg	12/60	25	
3. 2.0 mg	5/20	25	
4. 4.0 mg	3/20	15	
5. 0.5 mg	20/20	100	5 vs 1: N.S.*
6. 1.0 mg	19/20	95	6 vs 2: $P < 0.001$
7. 2.0 mg	13/20	65	7 vs 3: $P < 0.02$
8. 4.0 mg	2/20	20	8 vs 4: N.S.
9. 0.5 mg	8/15	53	9 vs 13: $P < 0.005$
10. 1.0 mg	—	—	
11. 2.0 mg	0/20	0	11 vs 15: $P < 0.05$
12. 4.0 mg	0/20	0	12 vs 16: N.S.
13. 0.5 mg	19/20	95	13 vs 5: N.S.
14. 1.0 mg	14/20	70	14 vs 6: N.S.
15. 2.0 mg	3/15	20	15 vs 7: $P < 0.01$
16. 4.0 mg	2/15	13	16 vs 8: N.S.

* N.S. = Not significant.

Mice were treated with either saline (1–8) or SZN (9–16) as for Fig. 1. 48 hr after the last injection they were given a series of injections of endotoxin in both saline (5–8) or SZN (13–16) treated groups. Animals in all cases were challenged with the indicated amount of endotoxin 48 hr after the last injection of the bacterial toxin.

of increasing doses of endotoxin induced a state of tolerance to the lethal effects of the toxin in saline pretreated mice. The establishment of tolerance to endotoxin was less pronounced in mice that had been pretreated with SZN than in the saline injected counterparts (see groups 5–8 vs 13–16 in Table 4). However, at each challenge dose of endotoxin, more animals died in the groups given SZN alone than in mice pretreated with both SZN and endotoxin (lines 9–12 vs 13–16, Table 4). This sort of impaired induction of tolerance was also evident when tolerance-inducing doses of endotoxin were given concurrently with those of SZN (Table 5). In other words, the combination SZN + endotoxin challenge manifested itself in a higher toxicity both in studies on acute lethality of (Tables 1 and 2), and tolerance to (Tables 4 and 5), endotoxin.

Data in Table 6 show that the rate of carbon clearance (K value), the total body weight, the spleen and the liver weights were all nearly identical in both the buffer and the SZN pretreated groups, albeit the rate of carbon clearance was higher than in entirely normal (untreated) mice (not shown). The K value, the spleen and the liver weights increased significantly as a result of endotoxin administration in the control group as in previous studies [1]. Endotoxin significantly increased the spleen weight and lowered total body weight in SZN pretreated mice (Table 6). Thus, SZN somewhat impaired the establishment of tolerance to endotoxin (Tables 4 and 5) but the

Table 5. Impaired tolerance to endotoxin lethality as a result of streptozotocin administration

Endotoxin	Living/ total	% Survival	Statistics
Buffer + E			
1. 0.5 mg	20/20	100	
2. 1.0 mg	15/15	100	
3. 2.0 mg	16/20	80	
SZN + E			
4. 0.5 mg	20/20	100	4 vs 1 N.S.*
5. 1.0 mg	20/20	100	5 vs 2 N.S.
6. 2.0 mg	6/20	30	6 vs 3: $P < 0.005$

* N.S. = Not significant.

Mice were injected with either saline or streptozotocin and increasing doses of endotoxin, concurrently, for five successive days, and challenged with the indicated dose of endotoxin 48 hr after the last injection.

Table 6. Influence of streptozotocin on various parameters of the reticuloendothelial system in mice

Treatment	*K* value	Body wt	Spleen wt	Liver wt
1. Buffer	0.046 ± 0.017 (6)	30.3 ± 1.36 (8)	91 ± 11 (8)	1526 ± 175 (8)
2. Buffer + endotoxin	0.079 ± 0.026 (7)	29.4 ± 0.98 (8)	206 ± 22 (8)	1733 ± 120 (8)
3. SZN	0.059 ± 0.009 (6)	28.6 ± 1.35 (8)	96 ± 19 (8)	1499 ± 107 (8)
4. SZN + endotoxin	0.078 ± 0.022 (8)	25.6 ± 2.02 (8)	204 ± 76 (8)	1602 ± 209 (8)
Statistics				
2 vs 1	$P < 0.02$	N.S.	$P < 0.001$	$P < 0.02$
3 vs 1	N.S.	N.S.	N.S.	N.S.
4 vs 3	N.S.	$P < 0.001$	$P < 0.001$	N.S.
4 vs 1	$P = 0.01$	$P < 0.001$	$P < 0.001$	N.S.

N.S. = Not significant. Mean ± standard deviation; sample size in parentheses.
Animals were injected with a combination of buffer or SZN with endotoxin as for Table 4. All determinations were carried out 48 hr after the last injection.

activation of the RES, that is usually associated with endotoxin administration, was largely unaffected. These data further undermine the role of the RES in the pathophysiology of endotoxicosis.

DISCUSSION

The results described here are consistent with earlier observations where endotoxin produced severe hypoglycemia in normal and hyperreactive animals [5, 8, 16]. Cortisol protection against endotoxin death, furthermore, was associated with only partial restitution of blood sugar. Therefore, the protective action of the hormone, at least in part, would appear to proceed via other host parameters. This is especially significant in view of the fact that triamcinolone acetonide, a molecule with 10 times the potency of cortisol, could not reverse blood sugar depletion by small sublethal doses of endotoxin. Nearly all organs are targets of glucocorticoid hormones which are known to affect so many processes in the cell that to point with any accuracy to the causal ones in endotoxicosis is hazardous indeed.

A unique feature of the studies described here is the sensitization to endotoxin lethality despite high blood sugar levels typical of the diabetic state following SZN administration. Whereas alteration in carbohydrate metabolism can be observed only after several days of SZN pretreatment (Fig. 1), sensitization to endotoxin lethality ensues immediately after an acute SZN load (Table 2). This is very intriguing since alloxan diabetes has not been reported to sensitize [16] whereas insulin increased endotoxin leathality [3]. Functional modification of the RES, too, seems to be ruled out as an explanation for hyperreactivity to endotoxin after SZN pretreatment (Table 6). Although used for selective β-cell destruction, SZN alters a wide variety of host processes that include immunosuppression [7], renal dysfunction [15], lipogenesis [9], liver enzymes [6], adrenal steroid metabolism [14] almost all of which are also influenced by endotoxin [2, 3]. However, these effects of SZN, just as elevated glycemia (Fig. 1), are observed only after several days or weeks whereas sensitization to endotoxin is concurrent with the administration of SZN. Thus, a more primary site of SZN action may yet have gone undetected. The possibility must also be kept open that endotoxin may sensitize to SZN although the LD_{50} of the drug for normal mice is unknown and the doses employed in this study induced no apparent toxic symptoms whatever.

In order to answer some of these questions several possibilities are at hand. A number of analogues of SZN, with varying degrees of toxicity, are available [17] and some of the aforementioned effects of SZN can be reversed by nicotinamide, pertussis vaccine [11], 3-*O*-methyl-D-glucose, antilymphocyte serum [13], and cortisone [18]. Whereas acute injections of nicotinamide or cortisone protect against lethality, vaccination with pertussis or chronic treatment with the hormone sensitizes mice to endotoxin [2, 3]. Thus, a study of the interaction between these agents, endotoxin, and SZN appears very promising in revealing aspects of host–parasite relationship.

REFERENCES

1. M. K. Agarwal, *J. Reticuloendothelial Soc.* **12**, 40 (1972).
2. M. K. Agarwal, *Die Naturwissenschaften* **62**, 167 (1975).
3. M. K. Agarwal, and G. Lazar, *Microbios.* **20**, 183, (1977).
4. G. Biozzi, B. Benacerraf, and B. N. Halpern, *Br. J. Exp. Pathol.* **34**, 441 (1953).
5. J. P. Filkins, in *Handbook of Shock and Trauma* (Eds. B. M Altura, A. Lefer and W. Schumer), Raven Press (1981).
6. A. Y. Chang, *Diabetologia* **13**, 595 (1977).
7. A. F. De Nicola, O. Fridman, E. J. Castillo and V. G. Foglia, *Hormone Metab. Res.* **9**, 496, (1977).
8. W. E. Farrar, and J. G. Watson, *Proc. Soc. Exp. Biol. Med.* **115**, 833 (1964).
9. V. Godbole, and D. A. York, *Diabetologia* **14**, 191 (1978).
10. L. B. Hinshaw, M. D. Peyton, L. T. Archer, M. R. Black, J. J. Coalson and L. J. Greenfield, *Surg. Gynecol. Obstet.* **139**, 851 (1974).
11. T. Katada, *Diabetologia* **13**, 521 (1977).

12. G. Lazar, and M. K. Agarwal, *Experientia.* **33**, 1359 (1977).
13. E. L. Lien, J. Greenwood and D. Sarantakis, *Diabetes* **28**, 491 (1979).
14. W. K. Nichols, J. B. Spellman, L. L. Vann and R. A. Daynes, *Diabetologia* **16**, 51 (1979).
15. R. Rasch, *Diabetologia* **16**, 125 (1979).
16. J. P. Filkins, L. W. Janusek and M. R. Yelich, in *Bacterial Endotoxins and Host Response* (Ed. M. K. Agarwal), Elsevier/North Holland (1980).
17. D. L. Vesley and G. S. Levey, *Hormone Metab. Res.* **10**, 392 (1978).
18. K. F. Wellmann and B. W. Volk, *Diabetologia* **13**, 331 (1977).