Induction of Insulin-dependent Diabetes by Streptozotocin

Inhibition by Estrogens and Potentiation by Androgens

S.-G. PAIK, M. A. MICHELIS, Y. T. KIM, AND S. SHIN

SUMMARY

Susceptibility of mice to experimental insulin-dependent diabetes as induced by multiple subdiabetogenic doses of streptozotocin has been shown to be strongly gender-dependent, males being much more susceptible than females. We examined this gender difference further in two strains of genetically susceptible mice to determine whether exogenous steroid sex hormones can both suppress the high susceptibility of males and potentiate the low susceptibility of females. Our results show that, in both BALB/cBOM and C57BL/6 mice, exogenous estrogens can suppress the high susceptibility of males. Conversely, the normally streptozotocin-resistant females become as highly susceptible as males after the administration of androgens. The inhibitory effect of estrogens and the potentiating effect of androgens can be demonstrated after the hormones are given to the mice either chronically (in slow-release capsules implanted at a subcutaneous site), or immediately prior to streptozotocin injection. These observations are consistent with the view that the critical factor that determines the susceptibility of mice to the hyperglycemic effects of streptozotocin is not the absolute concentration of androgens per se, but rather the relative overall level of androgens over estrogens in the recipient animal. Several alternative mechanisms for the effect of sex hormones on diabetogenic sensitivity are discussed.

DIABETES *31*:724–729, August 1982.

An elegant series of investigations by Like and Rossini and their co-workers demonstrated that streptozotocin, a methylnitrosourea derivative of 2-deoxy-glucose with selective toxicity to the pancreatic beta-cells, is a valuable experimental agent for the study of insulin-dependent diabetes mellitus.[1–3] The injection of a single high dose of this toxin (e.g., 200 mg/kg body wt/mouse) results in a rapid rise in the blood glucose level and a nearly complete destruction of the islets of Langerhans.[1] In contrast, the injection of the same total dose of streptozotocin divided into 5 subdiabetogenic doses (i.e., 40 mg/kg/day for 5 days) in genetically susceptible strains of mice leads to the induction of progressive hyperglycemia associated with the infiltration of the islets by lymphocytes ("insulitis").[1,2] While the hyperglycemic effects of the multiple dose treatment may be in part due to direct cytotoxicity of streptozotocin,[4] several lines of evidence suggest strongly that autoimmune mechanisms may also play an etiologic role.[1–3,5,6]

Curiously, the hyperglycemic action of streptozotocin is markedly influenced by the gender of the animal; males are, in general, significantly more susceptible than females.[6–8] In our own recent study using mice of the BALB/cBOM background, for instance, 100% of males given the multiple low dose injections of the toxin developed severe and permanent insulin-dependent hyperglycemia, but less than 15% of the females treated identically developed the syndrome.[6] A strong male preference has also been reported in the development of experimental diabetes in mice after infection by a diabetogenic virus,[9,10] and in rats treated with alloxan[11] or after a nearly total pancreactomy.[12,13] Recent epidemiologic data suggest that the incidence of type I diabetes in postpubescent children may also be higher in boys than in girls.[14] It seems possible, therefore, that the susceptibility to insulin-dependent diabetes, both in humans and in experimental animals, are influenced by common etiologic steps that are under the control of sex hormones.

In an initial study using mice of the noninbred strain CD-1, Rossini et al.[7] reported that castration of male mice led to decreased hyperglycemic response to multiple low doses of streptozotocin, and that this effect was reversed by testosterone treatment. Subsequently, MacLaren et al.[8] reported that female CD-1 mice showed an increased diabetogenic sensitivity to a single diabetogenic dose of the toxin if the mice were treated first with testosterone. Neither study, however, examined the effect of estrogen treatment on the susceptibility of genetic males, and these results together appeared to suggest that the major factor that determines the

From the Department of Genetics, Albert Einstein College of Medicine, Bronx, New York, and Department of Medicine, Cornell University Medical College, New York, New York.

Address reprint requests to Dr. Seung-il Shin, Department of Genetics, Albert Einstein College of Medicine, Bronx, New York 10461.

Received for publication 24 August 1981 and in revised form 13 May 1982.

sex difference may be simply the absolute level of androgens in the recipient animal regardless of the relative levels of estrogens. The purpose of our investigation was to test this hypothesis, by examining the effect of both androgens and estrogens in two strains of mice which had been shown to be highly susceptible to streptozotocin-induced hyperglycemia. We chose to use the multiple subdiabetogenic dose treatment with streptozotocin, since the delayed hyperglycemia induced by this procedure is likely to be a relevant model of the insulin-dependent diabetes in humans.[2,6]

MATERIALS AND METHODS

Mice. Mice were 6–8 wk old at the start of each experiment, and were either BALB/cBOM mice heterozygous for the *nu* allele, produced in our own nude mouse colony by mating homozygous nude (*nu/nu*) males with heterozygous (+/*nu*) females,[6] or C57BL/6J mice purchased from The Jackson Laboratory (Bar Harbor, Maine). To induce diabetes, mice were injected intraperitoneally with 40 mg/kg body wt of streptozotocin (mixed anomers, Sigma Co., St. Louis, Missouri) daily for 5 days.

Assay for plasma glucose levels. Blood samples were collected from the paraorbital venous plexus of nonfasting mice. Plasma glucose levels were measured as described previously.[6]

Implantation of slow-release sex hormone capsules. A long-term administration of sex hormone was achieved by subcutaneous implantation of slow-release steroid capsules. The steroid capsules were prepared as described by Seaman et al.[15] by placing 5 mg of either 17β-estradiol (Sigma) or 5α-androstan-17β-ol-3-one (5α-dihydrotestosterone) (Sigma) in 20-mm sections of 3-mm O.D. Silastic Medical Grade Tubing (Dow Corning Co., Midland, Michigan). The ends of the tubing were sealed with a wood pin using a medical adhesive (Silicone Type A, Dow Corning Co.). After shaving the hair from a small area on the back of the mouse near the tail, a 2–3-mm incision was made, and the capsule was implanted s.c. through the incision. The incision was closed afterwards with surgical glue (Histoacryl Blue, B. Braun, Melsungen, W. Germany).

Injection with solubilized hormones. Mice were injected subcutaneously with either testosterone enanthate U.S.P. (Delatestryl, Lot #9C635), or estradiol valerate U.S.P. (Delestrogen, Lot #7A821) (Squibb and Sons, Co., Princeton, New Jersey), after appropriate dilutions in sesame oil.

Assay for sex steroid hormone levels. Blood was collected from the heart by heparinized syringe, and the plasma was collected by centrifugation and stored at −20°C. Prior to assay, equal volumes of plasma from 2–4 mice for each group were pooled, and aliquots were assayed for estradiol and testosterone by radioimmunoassay (kindly performed by Dr. Lewis C. Krey, the Rockefeller University, and Dr. Benjamin Thysen, Albert Einstein College of Medicine).

Experimental design. Three experiments were designed based on different procedures of sex-hormone pretreatment. In the first, young adult mice of BALB/cBOM or C57BL/6J background were implanted with a Silastic capsule containing steroid hormone as described above. Male mice were implanted with a capsule containing 17β-estradiol, and females, with 5α-dihydrotestosterone. For each group, matching controls were implanted with a blank capsule. A month after the implantation, each of the mice was given daily i.p. injections of streptozotocin for 5 days (40 mg/kg body wt/day), and the blood glucose levels were determined at weekly intervals thereafter.

In the second experiment, we examined the effect of direct injections of solubilized sex hormones. Young adult BALB/cBOM mice were injected subcutaneously with steroid hormones solubilized in sesame oil at weekly intervals for 3 wk. Females were injected with testosterone (1.5 mg in 0.2 ml/mouse) and males with estradiol (0.09 or 0.9 mg in 0.2 ml/mouse). Controls were injected with sesame oil only. Beginning on the 10th day after the first hormone injection, all mice were treated with i.p. injections of streptozotocin for 5 days (40 mg/kg/day), and monitored for the development of hyperglycemia. The third experiment was similar to the second experiment, except that the mice were injected with either 0.05 or 0.5 mg of estradiol immediately (10 min) before each of the 5 daily injections of streptozotocin.

RESULTS

Suppression and potentiation of hyperglycemic response by long-term administration of hormones. We first examined the effect of chronic administration of sex hormones on hyperglycemia induction in BALB/cBOM and C57BL/6 mice. Young adult males were implanted with Silastic capsules containing 17β-estradiol, and young adult females with 5α-dihydrotestosterone. Control groups were implanted with empty capsules. In the estrogen-implanted males, the mean plasma level of estradiol eventually reached an eightfold higher level compared with the controls, while their testosterone level did not change significantly (Table 1). Similarly, the plasma testosterone level in the androgen-implanted females was elevated 2.5- to 8-fold over their controls, while their estradiol level again did not change very significantly.

Four weeks after the implantation, all mice were treated with multiple low doses of streptozotocin (40 mg/kg/day for 5 days), and examined for hyperglycemia induction as described in MATERIALS AND METHODS. All control males (not

TABLE 1
Plasma testosterone and estradiol levels in mice implanted with steroid hormone-containing capsules

Mouse strain	Sex	Hormone implanted	Mean hormone level (pg/ml)	
			Testosterone*	Estradiol
BALB/cBOM	M	None	880	26
	M	17β-estradiol	850	236
	F	None	100	61
	F	5α-dihydrotestosterone	260	97
C57BL/6J	M	None	260	<100
	M	17β-estradiol	360	>780
	F	None	<100	220
	F	5α-dihydrotestosterone	850	290

See MATERIALS AND METHODS for experimental details. Plasma steroid hormone levels were measured with plasma pooled from 2–4 mice per experimental group, 3 wk (for C57BL/6J) or 8 wk (for BALB/cBOM) after the implantation.
* Represents the total level of testosterone and 5α-dihydrotestosterone.

given estrogen) developed hyperglycemia by the second week after streptozotocin treatment, while most of the control females (not given androgen) did not develop comparable hyperglycemia even after the third week (Figure 1). These data confirm our earlier results obtained with BALB/cBOM mice.[6]

In contrast, estrogen-implanted males of both strains were uniformly resistant to streptozotocin (Figure 1, right half of panels A and C). Among the androgen-implanted females, a significant number (4/6) of BALB/cBOM mice became hyperglycemic (right half of panel B), but none of the C57BL/6 mice did so (right half of panel D). Whether the large differences in hyperglycemic response among the androgen-treated BALB/cBOM females reflected the differences in the estrous cycle was not examined.

These results demonstrate that the long-term administration of estrogen can fully suppress the hyperglycemic action of streptozotocin in normally susceptible males, and that androgen can potentiate it in normally resistant BALB/cBOM females.

Influence of pretreatment with solubilized sex hormones. To determine whether the incomplete "activation" of the females to the susceptible state by androgen implantation as seen in Figure 1 (B and D) is due to insufficient androgen concentration in the implanted mice, we next examined the effect of direct injections of solubilized hormones in young adult BALB/cBOM mice. Results of this series of experiments are summarized in Figure 2.

All (5/5) of the female mice given a relatively high dose of testosterone (1.5 mg/mouse/injection for 3 wk at weekly intervals) were found to be as susceptible as genetic males, in both the final incidence and the magnitude of hyperglycemia (Figure 2A). The establishment of streptozotocin-induced hyperglycemia was suppressed by estrogen in a dose-dependent manner (Figure 2B). The suppressive effect was clearly evident in mice injected with the lower dose of estrogen (0.09 mg/mouse/injection), but it was much more pronounced with the higher dose (0.9 mg) of estrogen used, so that all of the mice (6/6) of this latter group developed significantly less severe hyperglycemia. However, even the higher dose of estrogen used in this experiment was still insufficient to completely suppress the diabetogenic action of streptozotocin in the treated mice. Data in Figure 2 also show that the injection of relatively high dose of testosterone into genetic males, at a dose sufficiently high to fully "activate" the females (Figure 2A), did not significantly enhance the hyperglycemic response in the males (Figure 2B).

FIGURE 1. Effect of slow-release sex hormone implants on hyperglycemia response induced by multiple subdiabetogenic doses of streptozotocin. The steroid capsules were prepared and implanted s.c. as described in MATERIALS AND METHODS, **into BALB/cBOM mice heterozygous for the *nu* allele (A and B), or into C57BL/6J mice (C and D). Male mice (A and C) received either an empty capsule ("BLANK") or one containing 17β-estradiol ("β-ESTRADIOL"). Female mice (B and D) received either an empty capsule or one containing 5α-dihydrotestosterone ("DHT"). A month after the implantation, all of the mice were given daily i.p. injections of streptozotocin ("SZ") for 5 days (40 mg/kg body wt/day), and the plasma glucose levels were determined at weekly intervals thereafter according to the glucose-oxidase method as previously described (ref. 6).**

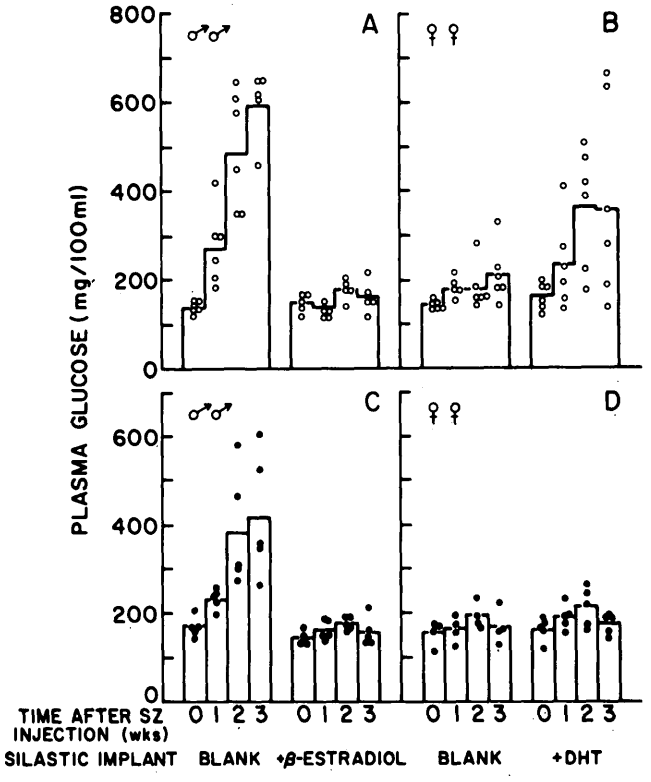
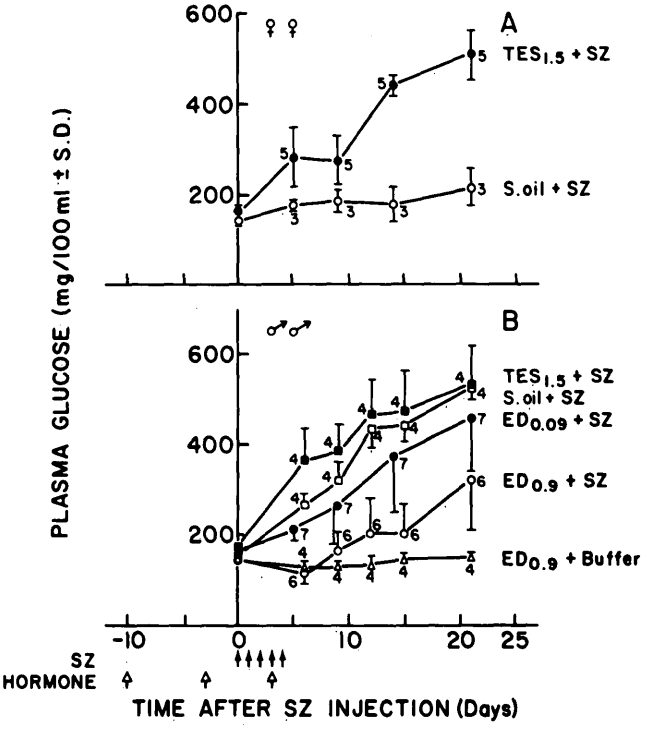


FIGURE 2. Effect of pretreatment of BALB/cBOM mice with testosterone ("TES") or 17β-estradiol ("ED") on induction of hyperglycemia with multiple subdiabetogenic doses of streptozotocin ("SZ"). Young adult mice (6–8 wk old) were injected s.c. with solubilized testosterone enanthate or estradiol valerate in sesame oil, on days −10, −3, and +3. Beginning on day 0, all mice were treated with i.p. injections of streptozotocin for 5 days (40 mg/kg/day), or with citrate buffer, and the plasma glucose levels were determined for 3 wk thereafter as indicated. The amount of steroid hormones injected per mouse at each treatment is given in mg. Control mice were injected with only the solvent, sesame oil. (A) females. (B) males. Vertical bars represent SD; the number next to each assay point refers to the total number of mice of that group used for the glucose determination.



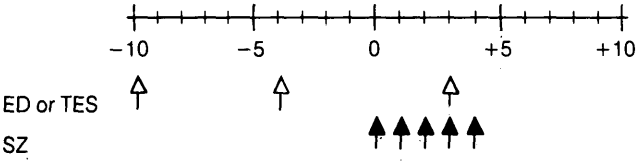
These observations demonstrate that the normally streptozotocin-resistant female mice can be made to be as susceptible as males if sufficiently high doses of exogenous testosterone are administered over a 3-wk period.

To assess the influence of exogenous androgen and estrogen on the steroid metabolism of the host, we examined the total plasma levels of sex hormones at various times after the treatment of BALB/cBOM mice with testosterone or with estradiol. We found that the mean testosterone level in the androgen-treated female mice was over 10 times the testosterone level of normal adult males (5120 versus 370 pg/ml) (Table 2). Similarly, the mean estradiol level in the estrogen-treated males was more than 50 times the estradiol level of normal adult females (1612 versus 20 pg/ml). Surprisingly, however, the treatment of females with testosterone resulted in a relatively insignificant elevation of estrogen levels, and the treatment of males with estrogen did not lead to a marked change in their total testosterone level. These results demonstrate that the administration of exogenous steroid hormones causes an effective reversal of the sex hormone balance of the mice.

TABLE 2
Plasma testosterone and estradiol levels in BALB/cBOM mice treated with solubilized hormones and streptozotocin

Sex	Treatment	Day* assayed	No. of mice	Plasma glucose (mg/dl ± SD)	Mean hormone level (pg/ml) Testosteronet	Estradiol
M	None	0	4	150 ± 34	370	18.1
	SZ only	+9	4	277 ± 49	550	24.6
	ED only	−4	4	125 ± 19	200	3375
	ED only	0	4	130 ± 9	180	1612
	ED + SZ	+4	3	97 ± 10	400	4947
	ED + SZ	+9	3	132 ± 5	235	2145
F	None	0	5	154 ± 18	25	20.2
	SZ only	+9	5	161 ± 21	107	29.7
	TES only	−4	4	133 ± 10	26480	61.5
	TES only	0	4	170 ± 15	5120	21.2
	TES + SZ	+4	4	129 ± 16	23500	41.1
	TES + SZ	+9	5	274 ± 53	7295	28.7

* Treatment and assay schedule:



Mice were injected with either estradiol valerate (ED; 0.9 mg/mouse) or testosterone enanthate (TES; 1.5 mg/mouse) on 3 occasions as indicated by open arrows. Control mice were injected with sesame oil only. Some of the mice were then injected with streptozotocin (SZ; 40 mg/kg/day) on days 0 to +4, as indicated by dark arrows. All mice were killed on days indicated to collect plasma. The plasma glucose level was determined with individual plasma sample, and the steroid hormone level was determined with plasma pooled from 2–3 mice. (The plasma samples of days −4 and +4 were collected 20 min after the injection of steroids.)
† Represents the total of testosterone and 5α-dihydrotestosterone levels.

Suppression of streptozotocin action by solubilized estrogens injected immediately prior to the toxin. The results presented so far showed that exogenous estrogen given in high enough doses beginning at least 10 days before the injection of streptozotocin could completely suppress the induction of hyperglycemia in genetically susceptible male mice. Since, as we discussed earlier, the hyperglycemic action of subthreshold doses of streptozotocin is likely to be mediated by autoimmune processes, it seemed probable that the suppressive effect of the long-term pretreatment with estrogen may be exerted through its inhibitory action on host immune functions. To approach this question, we now asked whether a long-term presence of excess estrogens prior to the injection of streptozotocin was in fact necessary.

BALB/cBOM males were injected with solubilized estrogens immediately prior to each of the 5 daily injections of streptozotocin, and the development of hyperglycemia was monitored during the following 3 wk. The results of this experiment, presented in Figure 3, show that the hyperglycemic action of streptozotocin is completely blocked by estrogens delivered immediately before the toxin, provided that a sufficiently high dose of hormone is used (Table 3). It is noteworthy that the effective suppressive dose of estrogen according to this procedure is considerably lower than the estrogen dose required for suppression when tested according to the long-term procedure described for Figure 2. Whereas 0.5 mg estradiol was completely inhibitory when injected immediately prior to each administration of streptozotocin, 0.9 mg of estradiol was not sufficient to fully block the hyperglycemia induction when given at weekly intervals at times removed from the injection of streptozotocin. Consistent with this observation, the actual plasma level of estradiol was found to be similar in both groups of estrogen-treated males on day 0, when the first of streptozotocin injections was given (1612 versus 3340 pg/ml, Tables 2 and 3).

FIGURE 3. Effect of 17β-estradiol ("ED") pretreatment of BALB/cBOM male mice on hyperglycemic response induced by multiple subdiabetogenic doses of streptozotocin ("SZ"). Young adult male mice were injected s.c. with estradiol valerate dissolved in sesame oil 10 min before each of the 5 daily injections of streptozotocin. Other details were the same as those in Figure 2.

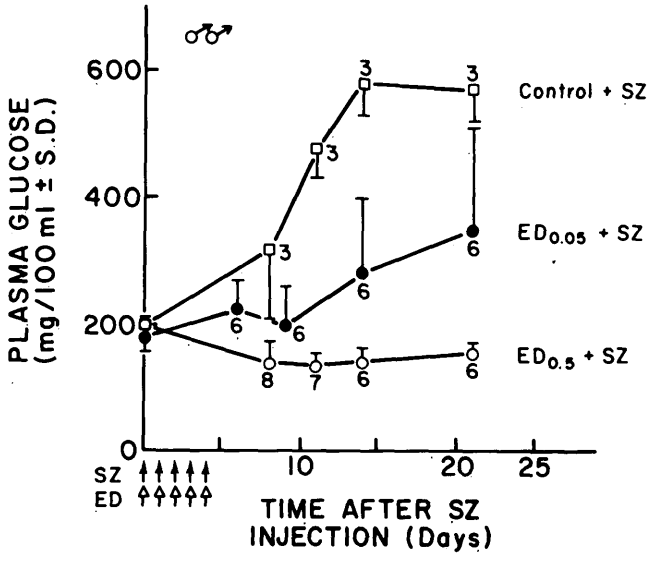
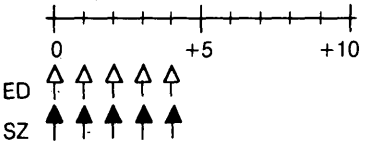


TABLE 3
Plasma testosterone and estradiol levels in BALB/cBOM male mice treated with solubilized estradiol immediately prior to streptozotocin injection

Treatment	Day* assayed	No. of mice	Plasma glucose (mg/dl ± SD)	Mean hormone level (pg/ml) Testosterone†	Estradiol
ED only	0	4	158 ± 26	411	3340
ED + SZ	+4	4	152 ± 24	755	3996
ED + SZ	+9	4	152 ± 22	420	7317

* Treatment and assay schedule:



Experimental details were similar to those described in the footnote to Table 2, except that the dose of estradiol valerate used in this experiment was 0.5 mg/mouse.
† Represents the total of testosterone and 5α-dihydrotestosterone levels.

DISCUSSION

These results demonstrate that exogenous sex hormones can fully reverse the susceptibility of mice to the hyperglycemic effects of streptozotocin. This reversal operates in both directions: the inherently high susceptibility of genetic males can be completely suppressed by estrogens, and the normally resistant genetic females can be made as highly susceptible as males by androgens. More specifically, the data shown in Figure 3 show that the sex hormone effect is very rapid, since the steroid hormones need not be present in excess in the recipient mice for long periods of time before the injection of streptozotocin. These observations, together with the data in Tables 2 and 3, strongly suggest that an overall relative excess of androgens over estrogens, rather than the absolute concentration of androgens, probably determines the streptozotocin sensitivity.

A marked sex dependence in susceptibility to streptozotocin-induced hyperglycemia was first described by Rossini et al.[16] In a brief report, they also showed that castration of CD-1 male mice decreased the hyperglycemic response to multiple low doses of streptozotocin, and that testosterone treatment could restore the response in castrated males and enhance it in normal and castrated females.[7] Subsequently, MacLaren and associates[8] reported that a similar sex difference in diabetogenic response existed in DBA/2J mice treated with a single high diabetogenic dose of streptozotocin. However, they were unable to demonstrate any inhibitory effect of estrogen pretreatment in uncastrated CD-1 male mice given a single diabetogenic dose of the toxin (80 or 120 mg/kg for this strain of mouse). Although the mechanism of diabetogenesis by the high diabetogenic dose of the toxin is probably quite different from that of the multiple subdiabetogenic doses which is believed to involve an autoimmune amplification mechanism,[1-3,6] these observations together seemed to support the conclusion that the striking sex difference in hyperglycemic sensitivity observed in both of these cases may be determined simply by the concentration of androgens per se, regardless of the estrogen levels in the recipient mice. Our results seem to rule out this possibility.

The results presented here confirm and extend the reports of Rossini and MacLaren and their collaborators to two additional strains of mice. However, our results are also significantly different from these earlier reports in at least two respects. Rossini's group[16] had reported that BALB/c mice were relatively resistant to the diabetogenic effect of multiple low doses of streptozotocin, in terms of the induction of both hyperglycemia and lymphocytic infiltration of the pancreatic islets ("insulitis"). Our data, on the other hand, demonstrate clearly that BALB/cBOM mice (which were originally derived from the BALB/c mouse) develop progressive hyperglycemia with a severity and high incidence comparable to CD-1 and B57BL/6J strains. In addition, we showed recently that the streptozotocin-induced hyperglycemia in BALB/cBOM mice is also associated with pronounced insulitis similar to what was previously reported for CD-1 mice by Rossini and co-workers.[17] Whether this apparent difference between the BALB/c substrains is due to unknown genetic variations between them, or to as-yet-unidentified environmental factors, remains to be seen. MacLaren et al.[8] reported that pretreatment of CD-1 male mice with 4 intraperitoneal injections of 0.005 mg estrogen over a period of 1 wk immediately prior to a single diabetogenic dose of streptozotocin did not significantly alter their diabetogenic sensitivity. Our data presented in Figures 2 and 3 suggest that their failure to demonstrate the suppressive effect of estrogen was probably due to the very low level of estrogen used. According to the procedure that we used, the optimal effective dose of estrogen for suppression of hyperglycemia induction is at least 0.5 mg (see Figure 3).

What is the basis of the sex difference? We may consider three major hypotheses. Firstly, estrogens could act indirectly, through their action on some components of the host immune system. This hypothesis is attractive, since a strong sex difference has been reported also for diabetes induced by certain viruses,[9,10] which may be mediated by an autoimmune mechanism, as well as for the incidence of juvenile-type diabetes in humans.[14] The main weakness of this hypothesis is that it fails to explain neither the strong sex difference observed in mice treated with high diabetogenic doses of streptozotocin (which presumably cause diabetes through direct physical destruction of the islet beta-cells), nor the inhibition of diabetogenesis by estrogens given immediately before the injection of multiple subdiabetogenic doses of streptozotocin. Secondly, estrogen excess might accelerate hydrolytic degradation of streptozotocin, by activation or induction of relevant enzymes in the animal. Streptozotocin is highly unstable, having a biologic half-life in vivo estimated to be about 5 min in mice.[18] Our own study indicates that there is no detectable difference between male and female BALB/c mice in the apparent half-life of streptozotocin in the plasma after intraperitoneal injection of the toxin (N. Fleischer, S.-G. Paik, and S. Shin, unpublished results). The rapidity with which injected estradiol can block the action of streptozotocin would also appear to rule out the induction of degradative enzymes as a potential mechanism. Thirdly, estrogens could block the action of streptozotocin directly, by interfering with the toxin's interaction with the target cell surface. It is reasonable to as-

sume that the diabetogenic effect of streptozotocin involves a selective interaction of the toxin with the islet beta-cells in the form of binding to the cell surface or cellular uptake, eventually leading to cellular damage. Glucose transport by some cell types is rapidly and reversibly inhibited by 17β-estradiol.[19] It is possible therefore that cellular uptake of streptozotocin, which is a derivative of glucose, is similarly inhibited by estrogens. Estrogen-dependent inhibition of streptozotocin transport by the islet beta-cells would naturally result in a reduction in diabetogenic effects of the toxin. If this hypothesis were correct, however, the sex difference in diabetogenesis induced by agents unrelated to streptozotocin must be based on alternative mechanisms.

ACKNOWLEDGMENTS

We wish to thank Dr. Benjamin Thysen, Dr. Lewis Krey, and Michael Gatz for performing the steroid assays, and André L. Brown for expert technical assistance.

This work was supported by research grants from the National Institutes of Health (AM26106) to S.S., and from the New York Diabetes Association to Y.T.K. S.-G.P. was supported by a Genetics Center Grant (GM19100) to Albert Einstein College of Medicine, and M.A.M. by the Lillia Babbitt Hyde Foundation and an NIH research grant (AI11694, to Dr. Gregory W. Siskind). S.S. is a recipient of a Faculty Research Award from the American Cancer Society and the Irma Hirschl Trust.

REFERENCES

[1] Like, A. A., and Rossini, A. A.: Streptozotocin-induced pancreatic insulitis: new model of diabetes mellitus. Science *193*:415–17, 1976.

[2] Rossini, A. A., Like, A. A., Chick, W. L., Appel, M. C., and Cahill, G. F., Jr.: Studies of streptozotocin-induced insulitis and diabetes. Proc. Natl. Acad. Sci. USA *74*:2485–89, 1977.

[3] Rossini, A. A., Williams, R. M., Appel, M. C., and Like, A. A.: Complete protection from low-dose streptozotocin-induced diabetes in mice. Nature *276*:182–84, 1978.

[4] Bonnevie-Neilsen, V., Steffes, M. W., and Lernmark, A.: A major loss in islet mass and B-cell function precedes hyperglycemia in mice given multiple low doses of streptozotocin. Diabetes *30*:424–29, 1981.

[5] Buschard, K., and Rygaard, J.: Is the diabetogenic effect of streptozotocin in part thymus-dependent? Acta. Pathol. Microbiol. Scand. C. *86*:23–27, 1978.

[6] Paik, S. -G., Fleischer, N., and Shin, S.: Insulin-dependent diabetes mellitus induced by subdiabetogenic doses of streptozotocin: obligatory role of cell-mediated autoimmune processes. Proc. Natl. Acad. Sci. USA 77:6129–33, 1980.

[7] Rossini, A. A., Williams, R. M., Appel, M. C., and Like, A. A.: Sex differences in the multiple-dose streptozotocin model of diabetes. Endocrinology *103*:1518–20, 1978.

[8] MacLaren, N. K., Neufeld, M., McLanghlin, J. V., and Taylor, G.: Androgen senitization of streptozotocin-induced diabetes in mice. Diabetes *29*:710–16, 1980.

[9] Boucher, D. W., Hayashi, K., Rosenthal, J., and Notkins, A. L.: Virus-induced diabetes mellitus. III. Influence of the sex and strain of the host. J. Infect. Dis. *131*:462–66, 1975.

[10] Morrow, P. L., Freedman, A., and Craighead, J. E.: Testosterone effect on experimental diabetes mellitus in encephalomyocarditis (EMC) virus infected mice. Diabetologia *18*:247–49, 1980.

[11] Goodman, M. N., and Hazelwood, R. L.: Short-term effects of oestradiol benzoate in normal, hypophysectomized and alloxan-diabetic male rats. J. Endocrinol. *62*:439–49, 1974.

[12] Houssay, B. A.: Action of sex hormones on experimental diabetes. Br. Med. J. *2*:505–10, 1951.

[13] Rodriguez, R. R.: Influence of oestrogens and androgens on the production and prevention of diabetes. *In* On the Nature and Treatment of Diabetes. Wrenshall, G. A., and Leibel, B. S., Eds. Amsterdam, Excerpta Medica Foundation, 1965, pp. 288–307.

[14] West, K. M.: Epidemiology of diabetes and its vascular lesions. Amsterdam, Elsevier, 1978, pp. 213, 330.

[15] Seaman, W. E., Blackman, M. A., Gindhart, T. D., Roubinian, J. R., Loeb, J. M., and Talal, N.: β-estradiol reduces natural killer cells in mice. J. Immunol. *121*:2193–98, 1978.

[16] Rossini, A. A., Appel, M. C., Williams, R. M., and Like, A. A.: Genetic influence of the streptozotocin-induced insulitis and hyperglycemia. Diabetes *26*:916–20, 1977.

[17] Paik, S. -G., Blue, M. L., Fleischer, N., and Shin, S.: Diabetes susceptibility of BALB/cBOM mice treated with streptozotocin: inhibition by lethal irradiation and restoration by splenic lymphocytes. Diabetes 1982. In press.

[18] Schein, P. S., and Loftus, S.: Streptozotocin: depression of mouse liver pyridine nucleotides. Cancer Res. *28*:1501–1506, 1968.

[19] Gay, R. J., and Hilf, R.: Paradoxical effects of 17β-estradiol on glucose transport in primary cell cultures of a rat mammary tumor. Biochem. Biophys. Res. Commun. *92*:1180–88, 1980.