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Urinary Excretion of Nitrogenous Compounds and Ketones in Streptozotocin Treated Rats *

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During the second and third day after the intraperitoneal injection of 65 mg/kg of streptozotocin to rats the daily urinary excretion of urea, ammonia and ketone bodies is significantly elevated over levels seen in control rats. The streptozotocin treated rats show polyuria but the excretion of urinary creatinine did not differ from that in the controls. Fasted animals showed no significant differences between streptozotocin treated animals and control groups in all parameters measured. The results have been related with the *in vivo* utilization of aminoacids in diabetes to maintain elevated liver and renal gluconeogenesis.

It is well established that gluconeogenesis is altered in diabetogenic situations (for a review see ref. 5). These studies have been carried out by providing enough substrates to «load» gluconeogenic mechanisms and thus to determine the maximal enzymatic potential to synthesize glucose. These experiments are unsatisfactory in accurately evaluating *in vivo* events as the animal depends on endogenous substrates to make glucose. This is especially true in the fasting state where the primary gluconeogenic substrates are the aminoacid products of protein catabolism. To asses the consequences of these changes in the diabetic animal we studied the urinary excretion of nitrogenous compounds in rats treated with streptozotocin. This N-nitrosourea derivative of glucosamine exerts its cytotoxic action on pancreatic β-cells (15, 16) leaving intact the α-cells and the exocrine pancreas (11, 14). We have previously shown that streptozotocin produces great changes in liver composition, blood glucose, ketone bodies and insulin concentrations in fed rats which are ameliorated in fasted animals (13). These considerations prompted us to carry out the study in both fed and fasted conditions.

* Part of this study was carried out at the Cátedra de Fisiología Animal, Facultad de Ciencias. Universidad de Madrid.

Materials and Methods

Male Wistar rats weighing 126-187 g and fed on a standard pellet diet were housed in individual metabolic cages for three days before the onset of the experiment. One group of animals was given a single i. p. injection (65 mg/kg) of streptozotocin *. The drug was injected within 5 min. after dilution in 0.5 ml citrate buffer (0.01 M, *p*H 4.5). Controls were injected with 0.5 ml of citrate buffer. Urine was collected for 24-h periods during the second and third day after the injections. During this time, half of the animals in each group were fed *ad libitum* while the other half were fasted, allowing free access to drinking water. Specimens were collected under light mineral oil in flasks containing 0.5 ml 6N HCl. The urine was processed as decribed elesewhere (9). Urinary urea, ammonia, creatinine and ketone bodies were evaluated by colorimetric methods (1, 2, 4, 6). Recovery experiments to determine possible losses of ketones into mineral oil were carried out as previously described (10).

* Kindly supplied by Dr. W. E. Dulin, The Upjohn Co., Mich., USA.

Results

The treatment with streptozotocin produces an intense diabetes in rats as manifested by their elevated blood glucose and decreased plasma insulin concentrations (13). Fed rats show significant elevations in urine volumes excreted on day 2 and 3 after the injection of 65 mg/kg of the drug (Table I). The amount of urine excreted is reduced in fasted control animals. The streptozotocin induced elevation in urine volumes seen in fed rats is prevented by fasting the animals (Table I). Similar results are found in the other parameters studied with the exception of the urinary excretion of creatinine. Thus, when the animals are fed, the daily excretion of urea, ammonia and ketone bodies are significantly higher in the streptozotocin treated animals than in the controls. All these parameters are lower in the fasted state, but the fall is greater

Table I. *Effect of streptozotocin on 24-h urinary excretion of nitrogenous compounds and ketones in fed and fasted rats.*
Rats received a single intraperitoneal injection of streptozotocin (65 mg/kg). The results are given as means ± SEM of 5-8 animals/group. P, corresponds to the differences between each streptozotocin group and its respective control. NS, not significant, i.e. $P > 0.05$.

Day after the injection of the drug:	2			3		
	Control	P	Streptozotocin	Control	P	Streptozotocin
	Dietary Status: Fed					
Urine (ml)	13.8± 1.2	< 0.01	44.9± 7.4	15.4± 1.4	< 0.001	53.1± 6.4
Urea N (mg)	108±21	< 0.05	337±36	85± 9	< 0.001	321±12
Ammonia N (mg)	34.2± 5.7	< 0.001	114±62	43.0± 5.9	< 0.02	80.0± 9.5
Creatinine N (mg)	6.3± 0.5	N.S.	8.4± 1	9.2± 1.8	N.S.	12.3± 1.7
Ketones (μmoles)	116±13	< 0.05	184±25	124±14	< 0.05	263±45
	Dietary Status: Fasted					
Urine (ml)	9.7± 0.5	N.S.	13.5± 2.0	5.3± 0.5	N.S.	9.8± 1.8
Urea N (mg)	106± 8	N.S.	70±23	71± 5	N.S.	62±12
Ammonia N (mg)	24.0± 4.1	N.S.	40.3± 7.4	20.4± 3.1	N.S.	29.5± 4.5
Creatinine N (mg)	4.5± 1.7	N.S.	4.7± 0.4	3.2± 0.2	N.S.	3.4± 1.0
Ketones (μmoles)	18± 3	N.S.	38±12	26± 7	N.S.	16± 4

in the streptozotocin treated rats than in the controls the differences between both groups (Table I) thus disappearing.

The differences between the diabetic animals and the controls could be simply due to changes in the glomerular filtration rate. To have an indirect index of this we have measured the urinary excretion of creatinine in the different experimental groups. As shown in Table I, streptozotocin treatment in fed and fasted animals has not effect on urinary creatinine. Urinary creatinine decreases with fasting in both streptozotocin treated and control rats (Table I) and this agrees with previous observations (10).

Discussion

We have seen in the present study that rats fed *ad libitum* and treated with streptozotocin show intense polyuria as an index of their severe diabetes. This is consistent with our previous findings of hyperglycemia and hypoinsulinemia in animals under similar experimental conditions (13). All of this confirms studies by others (11, 14-16) showing the diabetogenic action of this drug.

The elevated excretion of nitrogenous conpounds in the fed rats treated with streptozotocin suggest that the catabolism of proteins in these animals is intense. This would fit in with an elevated utilization of aminoacids for gluconeogenesis. Actually gluconeogenesis is elevated in diabetogenic conditions (5) and we have previously demonstrated that the livers of streptozotocin treated animals have the proper steady state concentration of regulatory metabolites to have augmented gluconeogenesis in the fed state (13). The fact that the concentration of urea in the urine is elevated in the streptozotocin treated animal supports this view of enhanced liver gluconeogenesis as it is well known that the end product of the aminoacid convertion to gluconeogenic metabolites in the liver is ammonia which is rapidly converted to urea by the Krebs-Henseleit cycle before being excreted.

The elevated urinary excretion of ammonia in the fed streptozotocin treated rats suggests that renal gluconeogenesis is elevated in these animals, as there may exist a coupling of renal ammoniogenesis and gluconeogenesis as han been demonstrated in other experimental situations (3, 7, 8).

The augmented ammoniuria in the streptozotocin treated animals coincides with an elevation of urinary ketone bodies. This is consistent with the hyperketonemia previously described in this laboratory (13). Actually the acidosis may trigger both the activation of renal ammonia and glucose production in these animals, as has been demonstrated under different experimental conditions (3, 7, 8, 10, 12).

The metabolic picture is more difficult to analyze in these animals when food is withheld. The rise in urea, ammonia and ketone bodies seen in fed rats treated with the drug vs. controls does not occur in fasted animals. The polyuria is no longer present in fasted animals and the streptozotocin treated rats are not significantly different from controls. All of these results agree with the lack of differences observed between streptozotocin treated animals and their controls when fasted in the steady state concentration of liver metabolites and the decrease of the whole diabetogenic picture observed (13). Although further experimental suport is required to interpret all these findings, they might be partially explained by one the two following possibilities: *a*) Diabetes is milder in these animals when fasted and the reduction of the factors which drive their metabolic disturbances in the fed state facilitates the normalization of these parameters, or *b*), the endogenous comsumption of these metatolites is augmented in the fasted diabetic animals and this does not allow

their accumulation even in the urine. Further studies are required to decide which of these hypothesis is correct.

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Resumen

La excreción urinaria de urea, amoníaco y cuerpos cetónicos en la rata está aumentada durante el segundo y tercer día de la inyección intraperitoneal de 65 mg/kg de streptozotocin. Estos animales presentan poliuria, pero la excreción urinaria de creatinina no difiere de la de sus respectivos controles. En ayunas, la cantidad de orina excretada diariamente por las ratas tratadas con streptozotocin y su contenido en urea, amoníaco y cuerpos cetónicos decrece más que en los controles, desapareciendo las diferencias entre ellos. La excreción urinaria de creatinina permanece igual en los dos grupos en ayunas. Los resultados se han relacionado con la utilización *in vivo* de aminoácidos para mantener elevadas la gluconeogénesis hepática y renal en el diabético.

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