Resistance of Rabbits and Guinea Pigs to the Diabetogenic Effect of Streptozotocin

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SUMMARY

Streptozotocin is an antibiotic with antitumor properties. It has been reported to be diabetogenic in dogs, monkeys, hamsters, rats, and mice. Rabbits and guinea pigs were found to be resistant to the diabetogenic action of streptozotocin. Resistance in these species was not related to impaired ability to maintain adequate serum concentrations of the drug. Streptozotocin incubated with rabbit serum and liver homogenate maintained its diabetogenic properties when injected in the rat. Liver and pancreas homogenates of the resistant species did not metabolize the drug in vitro as determined spectrophotometrically. It is postulated that the diabetogenic action of streptozotocin may be related to its ability to impair NAD synthesis. It is postulated that the drug apparently blocks the synthesis of NAD from nicotinamide but has no effect on the synthesis of NAD from nicotinic acid, the pathway which may be preferentially employed by rabbits and guinea pigs. DIABETES *18*:542-44, August, 1969.

Streptozotocin is a broad spectrum antibiotic.[1] It has been shown to have antitumor property against several experimental tumors[2] and recently it has been used in the treatment of a malignant islet-cell tumor in a human.[3] Several authors have reported that streptozotocin is diabetogenic in dogs, mice, rats, monkeys, and hamsters.[4-6] We recently found rabbits to be resistant to the diabetogenic action of streptozotocin.[7] As discussed later, we predicted and confirmed in the present study that guinea pigs are also resistant to this drug.

The present study was designed to elucidate the mechanism of the rabbit and guinea pig resistance to streptozotocin.

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METHODS, MATERIALS AND RESULTS

Experiment 1: Eight animals of each type—Sprague Dawley rats (450 gm.), New Zealand albino rabbits (2-3 kg.), and Hartley guinea pigs (250 gm.), were used.

A 2 per cent solution of streptozotocin* (U-9889 lot number 4621-HKY-81A) was prepared in 0.1 M citrate buffer pH 4.0. Five animals of each type received intraperitoneally a dose of 130 mg./kg. streptozotocin which is twice the diabetogenic dose in rats. Three animals of each type received 65 mg./kg. streptozotocin intraperitoneally.

Blood was drawn at 0, 15, 30, 45, and 90 minutes after drug administration and analyzed for streptozotocin concentration according to the method of Forist.[8]

Serum glucose was determined according to the method of Lowry[9] before drug administration and two and seven days after injection.

Before and following the streptozotocin administration, the animals were allowed to eat freely on Rockland rat/mouse diet, Rockland rabbit ration, and Rockland pig diet. These diets do not differ greatly in tryptophan, nicotinic acid, or nicotinamide.

The results are summarized in tables 1 and 2.

The data indicate that serum streptozotocin levels were similar in the three species. However, while all the rats became diabetic within forty-eight hours, none of the guinea pigs or rabbits showed an elevation of the nonfasting blood glucose.

Experiment 2: Since streptozotocin is reportedly concentrated in the liver of all species previously investigated[1] and since it exerts a direct effect on the pancreas,[5] an investigation was made into the ability of these organs to metabolize streptozotocin in vitro. Liver and pancreas were removed from rabbit, rat and guinea

*Kindly supplied by The Upjohn Co., Kalamazoo, Michigan.

TABLE 1

Blood sugar (mg./100 ml.) after intraperitoneal streptozotocin injection

Animals	Dose mg./kg.	Zero hours Mean	Zero hours Range	Forty-eight hours Mean	Forty-eight hours Range	Seven days Mean	Seven days Range
Rat	65	137	122-153	420	360-480	415	365-465
	130	135	125-145	432	374-490	430	387-473
Guinea pig	65	115	105-125	119	113-123	117	110-124
	130	116	107-124	117	113-121	118	111-125
Rabbit	65	127	116-137	126	109-145	120	106-134
	130	126	114-139	128	108-147	120	105-135

pig under ether anesthesia. The organs were kept at 2° C. while homogenized in a minimum amount of saline, and then centrifuged for ten minutes at 3,000 rpm. A known amount of streptozotocin was added to the supernatant to make a final concentration of 15 μg./ml. The pH of the liver mixture was 6.7 in the rat, 6.6 in the guinea pig, and 7.0 in the rabbit. The pH of the pancreas mixture was 6.0 in all animals. The same amount of drug dissolved in 0.1 M. citrate buffer of the same pH served as control. All mixtures were incubated in a water bath for fifteen minutes at 37° C. The drug concentration was then determined as mentioned previously. In all instances, the amount of drug present was not less than 99 per cent of the concentration before incubation. As the in vivo action of the drug is quite rapid,[6] these data suggest that the resistance of rabbits and guinea pigs to streptozotocin cannot be attributed to inactivation of the drug in the liver or the pancreas. To confirm this hypothesis streptozotocin incubated for thirty minutes with rabbit serum and rabbit liver homogenate was injected into five rats at a dose of 65 mg./kg. All rats became diabetic within forty-eight hours.

DISCUSSION

The possibility that rabbits and guinea pigs fail to develop diabetes after streptozotocin administration because of the inability to attain sufficient blood levels of the drug, due either to poor absorption or rapid inactivation, would not seem to be borne out by our data. High levels of streptozotocin were found in the serum of both resistant species. It was also demonstrated that the concentration of streptozotocin did not decrease after in vitro incubation with rabbit's and guinea pig's liver and pancreas homogenates. Unfortunately, the method of determining streptozotocin measures only the N-nitroso group on the urea portion of the streptozotocin side chain and cannot be considered proof of the presence of a diabetogenic compound. However, streptozotocin incubated with rabbit serum and rabbit liver homogenate remained diabetogenic in rats. It would appear from these results that the resistance of rabbits and guinea pigs to streptozotocin is not due at an unusually rapid rate of inactivation.

The possibility must be considered that the specific pathway upon which the drug exerts its diabetogenic effect may be different in various species, thereby rendering some species resistant.

It has been shown that streptozotocin causes nicotinamide adenine dinucleotide (NAD) depression in the liver and that streptozotocin probably impairs NAD synthesis.[5] Two main pathways have been elaborated for the synthesis of NAD:[10,11]

1. Nicotinamide ⟶ NAD.
2. Nicotinic acid ⟶ NAD.

A third pathway of NAD synthesis from tryptophan[12,13] has been established recently, but is found

TABLE 2

Streptozotocin levels (μg./ml.) in serum after intraperitoneal streptozotocin injection

Animals	Dose mg./kg.	Time after injection: Fifteen minutes Mean	Fifteen minutes Range	Thirty minutes Mean	Thirty minutes Range	Forty-five minutes Mean	Forty-five minutes Range	Ninety minutes Mean	Ninety minutes Range
Rat	65	47	42-52	55	52- 58	48	46- 50	4.0	2.4-5.6
	130	90	81-99	110	101-119	95	89-101	34	29-39
Guinea pig	65	46	42-50	52	49- 55	49	46- 52	4.0	2.8-5.2
	130	89	82-96	109	100-118	100	89-111	25	21-29
Rabbit	65	49	46-52	56	53- 59	49	46- 52	4.0	2.4-5.6
	130	89	83-95	108	103-113	104	98-110	30	24-36

only in liver and kidney.

Dogs and rats which are susceptible to the diabetogenic action of streptozotocin tend to aminate most of their nicotinic acid to form nicotinamide.[14] From this it could be inferred that these species use the nicotinamide ——→ NAD pathway. Conversely it was found that rabbits and guinea pigs both tend to deaminate nicotinamide to form nicotinic acid[14] and it might be supposed that these animals use the nicotinic acid ——→ NAD pathway.

Nicotinamide administration prevents mice and rats from becoming diabetic after streptozotocin injection but nicotinic acid does not prevent the diabetogenic action of the drug.[5]

These observations led us to postulate that streptozotocin blocks the synthesis of NAD from nicotinamide but has little or no effect on NAD synthesis from nicotinic acid.

Based on the above reasoning we predicted that guinea pigs should fail to develop streptozotocin-induced diabetes. We found, indeed, that guinea pigs, as rabbits, resisted becoming diabetic even after 300 mg./kg. streptozotocin administration.

The diabetogenic effect of streptozotocin is due to damage of the pancreatic beta cells.[5,15] The diabetes caused by a single dose of streptozotocin is permanent while the depression of NAD content of the liver is reversible.[16] The question arises whether these two effects are related or distinct phenomenon. Schein and Loftus[16] postulated that the lowering of beta cell NAD over a critical period of time might irreversibly destroy the beta cell. Investigations of the effect of streptozotocin on the NAD content of the beta cells appear warranted.

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