DISTRIBUTION OF ^{14}C AND ^{3}H-STREPTOZOTOCIN IN DOGS AND TOADFISH

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Since streptozotocin shows a highly specific action on the function of islet cell tissue of the pancreas, a tissue distribution study of the labeled compound was completed to evaluate possible concentration of streptozotocin in the beta cells. Ten dogs and 46 toadfish were injected intravenously with ^{14}C- or ^{3}H-labeled streptozotocin. Tissue distribution studies were completed in dogs at 1, 2, 3, 4, 6, or 24 hr and in toadfish at 10 min, 1, 2, 3, 4, 6, or 24 hr.

No selective concentration of labeled streptozotocin occurred in dog pancreas at any time interval. Islet cells of the toadfish also failed to show specific concentration of streptozotocin. There was no significant difference in the pattern of distribution and excretion between both labeled compounds.

Streptozotocin is a 2-deoxy-D-glucose derivative of N-methyl-N-nitrosourea isolated from Streptomyces achromogenes. It is an antibiotic active against a variety of organisms (*1,2*).

The compound also has antitumor activity in leukemia L5178 and a few carcinomas (*3*). Rakieten, et al (*4*) reported in 1963 that single intravenous or large dose administration of streptozotocin produced frank diabetes mellitus in dogs and rats. The compound has since become more widely known as a diabetogenic drug. After streptozotocin was demonstrated to produce degranulation of the beta cells of the pancreas (*3,5*) the compound became appealing as a promising agent for the treatment of functioning beta cell carcinoma of the pancreas.

In 1968, Murray-Lyon, et al (*6*) administered streptozotocin for the first time to a patient with malignant islet cell tumor of the pancreas. Excellent symptomatic relief and striking regression of the metastasized tumor mass followed. Sadoff (*7*) also reported a case of successful treatment of islet cell carcinoma of the pancreas with intravenous streptozotocin. Many investigators have since confirmed these results in other patients with metastatic beta cell carcinoma of the pancreas.

Although inhibitory effects of streptozotocin on some oxidative enzymes (*8*), on DNA synthesis (*9*), and on nicotinamide adenine dinucleotides (*10*) are reported, the exact mode of the diabetogenic action of streptozotocin is not yet clarified. Since the streptozotocin shows a highly specific action on the function of islet cell tissue of the pancreas, we have explored the possibility that the radiolabeled compound might be concentrated in the beta cells.

METHOD

Radioactive compound. The chemical structure of the ^{3}H- and the ^{14}C-labeled streptozotocin is presented in Fig. 1. The ^{14}C- or ^{3}H-labeled streptozotocin was provided by the Monsanto Research Corp., Dayton, Ohio, on contract through the National Cancer Institute. Radiochemical purity was established with TLC using silica gel G as the absorbent and a solvent system of ethanol:benzene (1:2). The stability of streptozotocin in aqueous solutions has been determined colorimetrically with polargraphy and by bioassay (*11*). Specific activities of the two compounds were as follows: ^{14}C-streptozotocin, 6.5 mCi/m*M*; the ^{3}H-streptozotocin, 37.5 mCi/m*M*. The compound was placed in a desiccator and kept in the freezer. It was dissolved with 0.05 *M* citric acid butter, pH 4.5, about an hour before use.

Dogs. Studies were performed in ten mongrel dogs of either sex weighing 9–12 kg. Each dog was given an intravenous tracer dose of ^{14}C- or ^{3}H-streptozotocin, 50 μCi, and sacrificed by rapid injection of in-

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STREPTOZOTOCIN

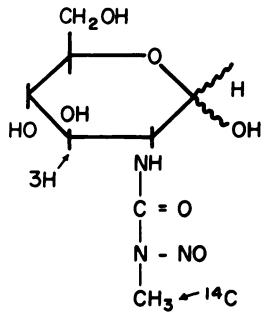

FIG. 1. Chemical structure of ^{3}H- or ^{14}C-labeled streptozotocin.

travenous lethal solution (pentobarbital sodium: 3.75 gm/cc, isopropyl alcohol 10.5%, propylene glycol 10.5%) at 1, 2, 3, 4, 6, or 24 hr after the dose. Blood was sampled from the heart chamber and two to four pieces of tissue were dissected from each organ or tissue for radioactivity assays.

Toadfish. Studies were performed in 46 toadfish (Opsanus tau). The toadfish islet cell tissue is segregated into one or more discrete bodies located in the mesentery and is relatively free from acinar tissue (*12,13*). Mature toadfish of either sex weighing 0.5–1.0 kg were kept in circulating artificial sea water. The tracer dose of ^{14}C- or ^{3}H-streptozotocin was injected into a gill arch vein through a 26-gage, 1-in. needle. Three to five fish were sacrificed by a blow on the head at 10 min, 1, 2, 3, 4, 6, or 24 hr after the tracer injection. Immediately after sacrifice, the ventral chest wall was opened and all possible blood was aspirated from the heart chamber. The major islets of the pancreas, usually one but occasionally two, were removed from the superior mesentery. Islet cell tissue was isolated from acinar tissues by means of dissecting the capsule from the 5 or 10 mg of islet cell tissue. Two to 4 mg of acinar tissue (part of capsular connective tissue included) were thus obtained. Two to three pieces of tissue samples weighing about 50 mg each were obtained from each organ or tissue for radioactivity distribution assay.

Radioactivity measurement. All specimens were placed in liquid scintillation counting vials, digested overnight in 0.3 ml of 10% NaOH, dissolved by warming to 70–80° for 20–30 sec, and after cooling, 3 drops of 30% H_2O_2 were added. Then 10 ml

TABLE 1. DISTRIBUTION OF ^{14}C- AND ^{3}H-STREPTOZOTOCIN IN DOGS

	Concentration of radioactivity in percent administered dose per gram tissue					
	1 hr	2 hr	3 hr	4 hr	6 hr	24 hr
Blood	0.02	0.01 (<0.01)*	0.02	0.02 (0.01)	0.01	0.01
Pancreas (head)	0.05	0.02 (<0.01)	0.04	0.07 (<0.01)	0.02	0.02
Pancreas (tail)	0.02	0.01 (<0.01)	0.05	0.07 (<0.01)	0.03	0.02
Liver	0.21	0.11 (0.03)	0.19	0.14 (0.03)	0.09	0.07
Renal cortex	0.10	0.05 (0.01)	0.09	0.09 (0.03)	0.06	0.06
Renal medulla	0.05	0.03 (<0.01)	0.05	0.07 (0.02)	0.02	0.01
Bladder	0.04	0.08 (<0.01)	0.02	0.13 (<0.01)	0.01	0.02
Lung	0.01	0.01 (<0.01)	0.02	0.03 (<0.01)	0.01	0.01
Heart	0.01	0.01 (<0.01)	0.01	0.01 (<0.01)	0.01	0.01
Spleen	0.01	0.01 (<0.01)	0.02	0.02 (<0.01)	0.01	0.01
Fat	<0.01	<0.01 (<0.01)	<0.01	<0.01 (<0.01)	0.01	<0.01
Muscle	0.01	0.01 (<0.01)	0.01	0.01 (<0.01)	0.01	<0.01
Intestine	0.01	0.01 (<0.01)	0.01	0.05 (<0.01)	0.03	0.04
Bile	0.13	0.14 (0.01)	0.11	0.03 (0.02)	0.15	0.017
Urine	1.77	0.42 (0.42)	0.35	0.69 (0.78)	0.45	0.13
No. of animals	1	4	1	2	1	1

* Data in parentheses: result of ^{3}H-streptozotocin.

TABLE 2. RELATIVE TISSUE DISTRIBUTION OF ^{14}C-STREPTOZOTOCIN IN TOADFISH

	Concentration of radioactivity in percent administered dose per gram tissue						
	10 min	1 hr	2 hr	3 hr	4 hr	6 hr	24 hr
No. of Fish	3	3	3	3	5	3	3
Blood	0.47 (0.15)*	0.13 (0.02)	0.19 (0.02)	0.14 (0.01)	0.12 (0.02)	0.11 (0.04)	0.07 (0.01)
Kidney	0.32 (0.07)	0.49 (0.09)	0.88 (0.08)	0.45 (0.10)	0.73 (0.25)	0.57 (0.22)	0.32 (0.03)
Liver	0.08 (0.02)	0.09 (0.02)	0.13 (0.02)	0.20 (0.03)	0.15 (0.02)	0.24 (0.08)	0.19 (0.03)
Pancreas islet	0.10 (0.01)	0.10 (0.01)	0.07 (0.01)	0.18 (0.02)	0.08 (0.01)	0.11 (0.05)	0.08 (0.02)
Pancreas acinar	0.16 (0.04)	0.15 (0.02)	0.12 (0.01)	0.27 (0.04)	0.13 (0.04)	0.15 (0.06)	0.06 (0.02)
Heart	0.30 (0.10)	0.10 (0.02)	0.12 (0.01)	0.14 (0.02)	0.10 (0.01)	0.11 (0.04)	0.12 (0.02)
Urine	0.01 (0.00)	0.05 (0.02)	0.22 (0.04)	0.14 (0.01)	0.04 (0.01)	0.18 (0.07)	0.24 (0.03)
Bile	0.01 (0.01)	0.01 (0.00)	0.02 (0.02)	0.03 (0.01)	0.03 (0.01)	0.08 (0.03)	0.13 (0.04)
Muscle	0.06 (0.02)	0.05 (0.01)	0.06 (0.01)	0.06 (0.01)	0.04 (0.01)	0.05 (0.02)	0.02 (0.01)
Spleen	0.03 (0.01)	0.08 (0.01)	0.07 (0.01)	0.09 (0.01)	0.07 (0.02)	0.07 (0.02)	0.10 (0.01)
Stomach	0.03 (0.02)	0.10 (0.01)	0.06 (0.01)	0.12 (0.01)	0.07 (0.01)	0.07 (0.03)	0.03 (0.00)
Intestine	0.03 (0.01)	0.10 (0.01)	0.09 (0.03)	0.15 (0.02)	0.09 (0.02)	0.09 (0.03)	0.04 (0.01)

* Mean + s.e.

TABLE 3. RELATIVE TISSUE DISTRIBUTION OF ^{3}H-STREPTOZOTOCIN IN TOADFISH

	Concentration of radioactivity in percent administered dose per gram tissue						
	10 min	1 hr	2 hr	3 hr	4 hr	6 hr	24 hr
No. of fish	4	3	3	3	3	3	3
Blood	0.95 (0.06)*	0.37 (0.07)	0.47 (0.21)	0.65 (0.07)	0.28 (0.13)	0.38 (0.04)	0.19 (0.04)
Kidney	0.16 (0.09)	0.14 (0.03)	0.55 (0.27)	1.86 (0.05)	1.60 (0.86)	1.02 (0.19)	0.24 (0.09)
Liver	0.21 (0.06)	0.27 (0.02)	0.14 (0.07)	0.41 (0.02)	0.21 (0.11)	0.60 (0.15)	0.21 (0.04)
Pancreas islet	0.19 (0.07)	0.35 (0.17)	0.09 (0.05)	0.16 (0.01)	0.06 (0.02)	0.17 (0.09)	0.14 (0.03)
Pancreas acinar	0.52 (0.27)	0.32 (0.05)	0.10 (0.05)	0.19 (0.02)	0.15 (0.07)	0.14 (0.04)	0.15 (0.03)
Heart	0.40 (0.10)	0.25 (0.04)	0.41 (0.24)	0.44 (0.06)	0.15 (0.07)	0.36 (0.09)	0.36 (0.07)
Urine	0.00 (0.00)	0.17 (0.07)	0.01 (0.01)	0.60 (0.26)	0.19 (0.08)	0.44 (0.21)	0.22 (0.07)
Bile	0.02 (0.01)	0.01 (0.01)	0.01 (0.00)	0.07 (0.01)	0.04 (0.03)	0.04 (0.01)	0.20 (0.03)
Muscle	0.04 (0.03)	0.10 (0.05)	0.06 (0.03)	0.166 (0.06)	0.09 (0.04)	0.24 (0.07)	0.17 (0.03)
Spleen	0.06 (0.03)	0.15 (0.04)	0.07 (0.03)	0.29 (0.07)	0.08 (0.04)	0.17 (0.05)	0.24 (0.05)
Stomach	0.21 (0.09)	0.09 (0.03)	0.10 (0.09)	0.33 (0.06)	0.19 (0.08)	0.40 (0.10)	0.24 (0.06)
Intestine	0.11 (0.07)	0.29 (0.10)	0.14 (0.07)	0.32 (0.03)	0.12 (0.09)	0.51 (0.18)	0.20 (0.06)

* Mean ± s.e.

of the liquid scintillation mixture was added (*14*). After 3–12 hr of cooling and dark adaption in the counter, samples were counted for 10 min each in a liquid scintillation counter (Searle Radiographics Unilux IIA). Quenching was corrected using the channels ratio method and counting efficiency for ^{14}C or ^{3}H was approximately 80% or 40%, respectively. Data were expressed as percent administered dose per gram of fresh tissue.

RESULTS

Table 1 presents the relative tissue distribution in dogs of ^{14}C- and ^{3}H-radioactivity concentration from ^{14}C- and ^{3}H-labeled streptozotocin. Table 2 presents the same data for ^{14}C-streptozotocin and Table 3 for ^{3}H-labeled streptozotocin in toadfish.

Dogs. No selective concentration of labeled streptozotocin occurred in the pancreas at any time interval with either radiolabel. Generally, the highest concentration of radioactivity in the dog was in liver at all time intervals and the second highest concentration was in kidney. The urine appeared to be the principal route of excretion and the bile, the secondary pathway. The pancreas showed higher concentration than intestine up to 4 hr after the injection but became lower than intestine activity after 6 hr. The activity in the liver, kidney, and pancreas was approximately 20, 10, and 5 times higher than that of background tissues such as muscle at earlier time intervals up to 4 hr.

Blood level of the compound was already very low at 1 hr and declined slowly thereafter up to 24 hr. The urinary excretion of streptozotocin was rapid (1.8% dose/gm of urine at 1 hr). The radioactivity concentration in the urine fell to 0.12% dose/gm urine by 24 hr.

Essentially, there was no difference between ^{3}H-labeled and ^{14}C-labeled compound in the relative distribution in various tissues of the dog.

Toadfish. Tables 2 and 3 show the distribution of ^{14}C- and ^{3}H-streptozotocin in various tissues of the toadfish at various time intervals from 10 min to 24 hr after the intravenous injection. Both labeled compounds revealed essentially the same pattern of distribution and excretion.

The highest concentration of radioactivity was present in the kidney at all time intervals. The radioactivity in the circulating blood fell rapidly after 10 min and showed a gradual decrease in concentration thereafter up to 24 hr. The radioactivity in the acinar tissue was slightly higher than in islet cell tissue. The concentration of radioactivity in the liver and bile revealed a gradual increase with time, plateauing in the liver at 3–6 hr. The radioactivity in the urine became higher than the activity level in the blood or in any other tissue except the kidney 2 hr after the injection and the higher activity remained up to 24 hr after ^{14}C-streptozotocin.

DISCUSSION

Tissue distribution studies of ^{14}C- and ^{3}H-streptozotocin in dogs failed to produce any evidence of the agent accumulating in pancreas. Since islets of Langerhans in mammals constitute only 1–2% of the total pancreas (*14*) it was speculated that radioactive streptozotocin in the islet, even if it were heavily accumulated in islet tissue, might not be sufficient to detect on assay of whole pancreas tissue.

Islet cell tissue of the toadfish, however, also failed to show specific concentration of streptozotocin. Radioactivity from streptozotocin in islet tissue was usually lower than the activity in plasma and also lower than that in acinar tissue of the pancreas. Therefore, we have produced no data to show that streptozotocin given intravenously is selectively concentrated in the islet tissue. On the basis of our present data, the streptozotocin probably does not enter the islet cells but remains in the extracellular compartment.

Consequently, it is presumed that the diabetogenic effect of the streptozotocin is through its action at the surface of the beta cell membrane. The mechanism of alloxan-induced diabetes is proposed as "through the action of the drug at the surface of beta-cells" (*13,16*).

An alternative explanation would be that there is a concentration in a subcellular fraction of the beta cell which is too small a percent of the cell volume to produce a significant increment in gross tissue radioactivity.

The concentration of radioactivity in kidney, fivefold greater than in pancreas and threefold greater in liver than in pancreas, is of interest in view of the reported development of tumors in kidney and liver after chronic administration of streptozotocin (*17,18*).

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