DISTRIBUTION OF ^{14}C-DIPHENYLHYDANTOIN IN PANCREAS ISLET CELL AND OTHER TISSUES OF TOADFISH

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Tissue distribution of ^{14}C-diphenylhydantoin (^{14}C-DPH) in the toadfish, with particular reference to the pancreatic islet cells, was studied in 24 toadfish at intervals of 10 min to 72 hr. At 10 min after intravenous injection of the tracer dose of ^{14}C-DPH, radioactivity concentration was 8% dose/gm in pancreatic islet cells with a pancreatic islet cell-to-liver ratio of 20:1. This concentration and target-to-nontarget ratio is substantially higher than that achieved previously with selenomethionine. The concentration in the brain was 6.2% dose/gm at 10 min. The ratio of relative radioactivity concentration in islet cells to acinar tissue, blood, liver, and skeletal muscles was: 6, 7, 20, and 27, respectively. Thirty minutes to 2 hr after tracer was injected, radioactivity became highest in the brain and second highest in the liver.

Diphenylhydantoin (DPH) causes hyperglycemia in vivo in animals (*1*) and suppresses glucose-induced insulin release in the isolated rat pancreas (*2*). Goldberg and Sanbar (*3*), and Klein (*4*) described marked hyperglycemia, which caused hyperosmolar coma in patients receiving DPH.

The suppressive effect of DPH on the insulin response to glucose stimulation was recently well demonstrated in a healthy man (*5,6*). However, the mechanism of DPH effects on insulin response is not known.

Although work has been done on tissue distribution of DPH in animals and man (*7–9*), the concentration of DPH in islet cells of the pancreas has never been evaluated.

A continuing program at this institution to develop scanning agents for specific localization in tumors has resulted in the study of several compounds for pancreatic scanning (*10*). We report here the substantial concentration of ^{14}C-DPH in the islet cells of the toadfish pancreas with a favorable target-to-nontarget ratio at 10 min after the tracer dose.

METHOD

Radioactive compound. Radioactive carbon-labeled DPH [5,5-diphenylhydantoin-4-^{14}C, $(C_6H_5)_2$ CNHCONH^{14}CO] was purchased from New England Nuclear Corp., Boston, Mass. The specific activity of the compound was 4.65 mCi/m*M*. The compound had a radiochemical purity of greater than 99% as determined by thin-layer chromatography: absorbent, silica gel G; methanol/acetic acid (98.2) benzene/ethyl/acetate, (2:1). The product is formulated by wetting the powder with ethanol (10%) and then adding water for injection containing 40% propylene glycol and sufficient NaOH to adjust the pH to 10–13.

Toadfish. The toadfish (Opsanus tau) was chosen as the experimental animal because the islet cell tissue of the toadfish is segregated into one or more discrete bodies located in the mesentery with only a capsule of acinar tissue easily separable from the islet tissue (*11,12*).

Mature toadfish of either sex weighing about 1 kg were kept in circulating artificial seawater. The tracer dose of 1.3–5.0 μCi of ^{14}C-DPH was injected into a gill arch vein through a 26-gage needle. A minimum of three fish were killed at each of the following intervals by a blow on the head; 10 min, 30 min, 1, 2, 6, 24, 48, and 72 hr. Immediately the ventral chest was opened and all possible blood was aspirated from the heart chamber. The major islets of the pancreas, usually one but occasionally two,

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were removed from the superior mesentery. The islet cell tissue was isolated from the acinar tissue by dissecting the capsule from approximately 10 mg of the islet cell mass.

Two to 4 mg of acinar tissue (part of capsular connective tissue included) were thus obtained. Two aliquots of tissue samples weighing about 30 mg each were obtained with 12 other tissues for radioactivity distribution assays as shown in Table 1.

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° for 20–30 sec and, after cooling, three drops of 30% H_2O_2 were added. Then 10 ml of PCS solubilizer (liquid scintillation mixture) from Amersham/Searle Co., Chicago, Ill. were added. After overnight dark adaptation and cooling in the counter, samples were counted for 10 min each in a liquid scintillation counter (Searle Radiographics Unilux IIA). Quenching was corrected using the two-channels ratio method and counting efficiency for ^{14}C was approximately 50%. Data were expressed as percent given dose per gram of fresh tissue.

RESULTS

Table 1 presents the distribution of ^{14}C-DPH radioactivity in 13 different tissues of the toadfish over intervals of 10 min to 72 hr after the intravenous tracer dose. The highest radioactivity concentration at 10 min after the dose was in the islet cell tissue (7.8% dose/gm). The concentration in the brain was second highest (6.2% dose/gm). The ratio of radioactivity concentration in islet cell to acinar tissue, blood, liver, and skeletal muscle was 6.0, 6.8, 19.7, and 26.5, respectively. From 30 min to 2 hr after the dose, radioactivity concentration was highest in the brain. After this interval, the relative radioactivity concentration in the liver became higher than in all other tissues. Radioactivity in the pancreas, islet cell, and acinar tissue showed a second peak at 6 hr. After 24 hr, peak radioactivity concentration occurred in the liver.

DISCUSSION

Hine and Kozelka demonstrated in rabbits that the concentration of DPH after intravenous administration was highest in the liver with less concentration in the brain, kidneys, and blood (*7*).

Tissue distribution of DPH was also studied in rats by Noach, et al (*9*), and in rats and dogs by Dill, et al (*8*). Their findings corresponded with Hine and Kozelka in that the highest concentration of DPH was found in the liver. However, none of these investigators measured DPH concentration in the islet cell

TABLE 1. TISSUE DISTRIBUTION OF ^{14}C-DIPHENYLHYDANTOIN IN TOADFISH AT VARIOUS TIME INTERVALS AFTER I.V. TRACER DOSE

	Data expressed as % dose/gm ± s.e.m., fresh tissue [Time (hr)]							
Tissue	1/6	1/2	1	2	6	24	48	72
Islet cell	7.77 ±1.09	1.72 ±0.20	0.77 ±0.29	0.56 ±0.14	1.18 ±0.18	0.43 ±0.03	0.18 ±0.05	0.42 ±0.10
Acinar tissue	1.29 ±0.33	0.79 ±0.04	0.63 ±0.09	0.39 ±0.12	1.80 ±0.59	0.23 ±0.04	0.20 ±0.03	0.41 ±0.08
Blood	1.14 ±0.21	0.44 ±0.38	0.36 ±0.05	0.25 ±0.03	0.15 ±0.03	0.24 ±0.03	0.11 ±0.01	0.22 ±0.01
Liver	0.39 ±0.02	2.17 ±0.46	0.60 ±0.09	0.80 ±0.10	0.74 ±0.18	1.03 ±0.23	0.54 ±0.04	0.86 ±0.21
Brain	6.22 ±1.30	3.19 ±1.02	0.94 ±0.09	0.94 ±0.22	0.67 ±0.16	0.69 ±0.10	0.34 ±0.05	0.69 ±0.06
Kidney	3.38 ±0.52	1.76 ±0.20	0.87 ±0.12	0.61 ±0.05	0.66 ±0.08	0.87 ±0.16	0.38 ±0.06	1.00 ±0.19
Urine	0.02 ±0.01	0.30 ±0.04	0.01 ±0.00	0.02 ±0.00	0.11 ±0.03	5.63 ±2.58	3.02 ±0.74	3.63 ±1.32
Bile	1.12 ±0.06	0.23 ±0.02	0.04 ±0.00	0.19 ±0.07	0.52 ±0.18	7.20 ±2.31	4.05 ±0.97	4.61 ±1.98
Heart	2.09 ±0.36	1.21 ±0.13	0.48 ±0.02	0.34 ±0.08	0.46 ±0.14	0.52 ±0.08	0.34 ±0.35	0.58 ±0.04
Spleen	0.22 ±0.04	0.86 ±0.22	0.12 ±0.03	0.16 ±0.06	0.36 ±0.06	0.38 ±0.03	0.25 ±0.02	0.49 ±0.05
Stomach	0.13 ±0.04	0.17 ±0.22	0.04 ±0.03	0.11 ±0.06	0.18 ±0.06	0.35 ±0.03	0.17 ±0.02	0.25 ±0.05
Intestine	0.09 ±0.03	0.19 ±0.03	0.20 ±0.07	0.18 ±0.01	0.31 ±0.05	0.44 ±0.06	0.30 ±0.02	0.41 ±0.03
Muscle	0.29 ±0.05	0.42 ±0.12	0.20 ±0.07	0.13 ±0.03	0.22 ±0.04	0.30 ±0.04	0.17 ±0.01	0.34 ±0.09
No. of fish	7	7	4	3	3	3	3	3

or the acinar tissue of the pancreas. The rate of disappearance of DPH from tissues and the rate of excretion were faster in these earlier studies than in this present study. This difference may be accounted for by the difference in experimental animals. In addition, Dill found that maximum blood and tissue levels were reached more rapidly with smaller doses.

The liver is the major organ of metabolism of DPH and an oxidized form of DPH, parahydroxyphenylhydantoin, is excreted in the urine as a glucuronated form (*12*). Consequently, after 24 hr a high concentration of radioactivity from ^{14}C-DPH is found in the bile and urine.

Since DPH is one of the most widely used anticonvulsants, extensive studies have been published on its effects on the excitability of the nerve cells (*14,15*), and on its distribution and binding in brain tissue (*16–18*). In their study on ^{14}C-DPH distribution and metabolism in rats, Noach, et al described the high concentration of radioactivity in the saliva and assumed that DPH might also accumulate in the pancreas as an exocrine gland similar to the salivary gland. The second peak of radioactivity was shown to be due to reabsorption from the gut.

From the results of the present study, it is clear that the concentration of DPH radioactivity in the islet cell 10 min postdose is highest, being seven times higher than in the blood and 20 times higher than in the liver. The concentration of ^{14}C-DPH of 8% dose/gm is more than ten times the maximum concentration that we have achieved with ^{131}I-19-iodocholesterol in the successful imaging of the adrenal gland in the human (*19*). The pancreas-to-liver ratio of 20:1 is significantly higher than the pancreas-to-liver ratio of 8:1 achieved by Blau and Manske with ^{75}Se-selenomethionine in the successful scanning of the pancreas (*20*). If this concentration of ^{14}C-DPH in the islet cell of the pancreas and in the brain is confirmed in the dog, we will prepare a radioiodinated analog of DPH to evaluate the possibility that this gamma-emitting radiolabeled compound might be concentrated in the dog and man for diagnostic pancreas and brain scanning.

Elucidation of the mechanism of DPH-induced hyperglycemia was not the object of this study but accumulation of DPH at its highest concentration in the islet cells soon after the intravenous dose was administered might contribute to the inhibition of insulin response to glucose stimulation.

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