ACTION OF L-THYROXINE ON CELL PROLIFERATION IN THE RAT PANCREAS

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UDC 612.34.014.2:612.6]-06:612.441.018

Administration of L-thyroxine to rats (500 μg per rat weighing 150-160 g daily for two days) caused marked stimulation of proliferative activity in the epithelium of the pancreatic acini. The mean 24-hourly mitotic index was increased by 15 times, and the number of cells synthesizing DNA was increased by more than 20 times compared with the control. L-thyroxine caused no significant changes in the level of proliferative activity of the islet cells.

The stimulant effect of thyroid hormone on proliferative processes in the pancreas has frequently been described [1-3]. However, quantitative data for the action of thyroid hormone on mitotic activity of the pancreatic epithelium are virtually absent.

The present investigation was accordingly carried out to study the action of L-thyroxine on the level of DNA synthesis and on mitotic activity in the pancreatic epithelium of rats.

L-thyroxine is known to stimulate mitotic activity in many organs. This stimulation is associated not only with an increase in the number of cells in the proliferative pool, but also with some decrease in the duration of the S-period of the mitotic cycle [4].

EXPERIMENTAL METHOD

Two series of experiments were carried out on the rat pancreas. In both series male albino rats with a mean weight of 150-160 g were used. In series I, six rats received subcutaneous injections of 500 μg L-thyroxine daily for two days at 1 P.M.; the remaining 6 rats acted as the control. On the following days all the animals received five intraperitoneal injections each of thymidine-H^3 in a dose of 0.6 μCi/g body weight at 2 and 7 P.M., midnight, and 5 and 10 A.M. The specific activity of the thymidine was 1.4Ci/mmole. The rats were sacrificed at 11 A.M., 1 h after the last injection.

In series II, 36 experimental rats also received injections of 500 μg L-thyroxine daily for two days. At 7 A.M. next day all the experimental animals and the same number of control rats received an injection of 250 μCi thymidine-H^3 (equivalent to about 1.7 μCi/g body weight). These animals were sacrificed in groups of three control and three experimental animals at a time, 30 min and 1, 2, 3, 4, 6, 9, 12, 15, 18, 21, and 24 h after injection of thymidine-H^3.

The pancreases were fixed by Carnoy's method and autoradiographs prepared in the usual way (sections 5 μ thick, "type M" emulsion, exposure 20 days). Depending on the number of labeled nuclei and mitoses, between 5000 and 30,000 acinar cells were counted in each case. The index of labeled nuclei and the mitotic index were determined. In series I all the cells of the islets falling in the field of vision also were examined and the number of dividing cells and the number of cells synthesizing DNA were determined.

Laboratory of Cytology, Institute of Medical Genetics, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 72, No. 8, pp. 93-95, August, 1971. Original article submitted March 4, 1971.

TABLE 1. Indices of Proliferation in Rats of Series I

Group of expts.	Rat	Acinar cells			Islet cells		
		no. of nuclei counted	index of labeled nuclei (in %)	mitotic index (in ‰)	no. of nuclei counted	index of labeled nuclei (in %)	mitotic index (in ‰)
Control	1	25 800	0,03	0,00	935	3,10	3,2
			0,21	0,04	632	2,85	0,0
	2	25 800	0,24	0,00	274	7,30	0,0
	3	25 800	0,45	0,04	891	2,35	0,0
	4	25 800	0,12	0,00	546	4,03	0,0
	5	25 800	0,03	0,00	1 272	2,52	0,0
	6						
Mean		25 800	0,18± 0,06	0,013± 0,003	910	3,69± 0,79	0,5
Action of L-thyroxine	7	8 700	7,00	1,95	244	1,64	0,0
	8	5 220	7,53	3,45	308	4,22	0,0
	9	17 400	1,18	1,72	353	2,27	0,0
	10	8 700	3,45	3,33	202	1,98	0,0
	11	8 700	5,44	2,64	Few islet cells		
	12	17 400	1,44	1,03	833	0,36	0,0
Mean		11 000	4,34± 1,01	2,35± 0,39	388	2,09± 0,62	0,0

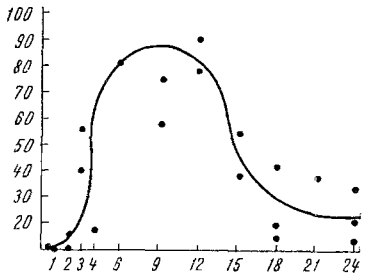

Fig. 1. Changes in percentage of labeled mitoses in epithelium of pancreatic acini of rats treated with L-thyroxine at successive times after injection of labeled thymidine. Abscissa, time after injection of thymidine-H^3 (in h); ordinate, percentage of labeled mitoses.

In series II, in cases in which more than 20 mitoses could be found, the number of labeled mitoses was calculated as a percentage of the total number of mitoses. A mitosis was regarded as labeled if it had three or more grains of reduced silver above it.

EXPERIMENTAL RESULTS

The results of the experiments of series I are given in Table 1.

The fact will be noted that, despite the five injections of labeled thymidine, revealing the total fraction of cells synthesizing DNA in the course of the 24-h period, the number of labeled acinar cells in the control rats was extremely small, namely 0.18%. There were likewise very few mitoses. In the islet cells the index of labeled nuclei was more than 20 times higher (3.69%) than in the acinar cells. Despite the fact that mitoses were found in the islet cells of only one rat, this could not indicate low proliferative activity, because in each case fewer than 1000 cells were counted, and the probability of finding a mitosis was low (for instance, if the mitotic index is 0.5‰, there is one mitosis to every 2000 cells).

Administration of L-thyroxine greatly stimulated proliferation in the acinar cells of the gland. The number of cells synthesizing DNA in the course of the 24-h period reached 4.3%, while the mitotic index rose to 2.4‰. However, this activation did not extend to the islet cells. The index of labeled nuclei in the experimental animals was slightly lower than in the control, although the difference was not statistically significant ($P = 0.14$).

The experiments of series II were carried out in order to discover whether the time taken by the cells to pass through the period of DNA synthesis (S) and through the postsynthetic period (G_2) is shortened when mitotic activity in the pancreas is stimulated by L-thyroxine.

However, in the control rats of this series, just as in the rats of series I, mitotic activity of the acinar epithelium was so low that in the overwhelming majority of animals the percentage of labeled mitoses could not be determined. In series II, the effect of L-thyroxine on the proliferation level could be judged in relation to two indices: 1) the mean index of labeled nuclei in the rats sacrificed during the first 4 h (the period of time when most cells taking up the label have not divided) which was 0.31± 0.07 in the con-

trol and 1.20 ± 0.28 ($P = 0.004$) in the experimental group; 2) the mean 24-hourly mitotic index, which was $0.11 \pm 0.04‰$ in the control and $1.50 \pm 0.31‰$ in the experimental series ($P = 0.000$).

Because of the increase in mitotic activity in the experimental rats, a curve of labeled mitoses could be obtained for these animals (Fig. 1). According to this curve the G_2-period for the experimental rats lasts about 31.5 h and the S-period about 11 h.

The relatively longer duration of the S-period in response to this degree of stimulation of proliferation may perhaps be attributable to two causes. First, the low initial level of proliferation, as judged from the control figures. Second, the insufficient time between the second injection of L-thyroxine and injection of thymidine. Mitotic activity in the experimental rats in fact continued to rise until the end of the experiment. For instance, in the 16 rats killed during the first 8 h after injection of thymidine (18-26 h after the second injection of L-thyroxine), the mean mitotic index was $0.61 \pm 0.13‰$, in the 8 rats sacrificed during the next 8 h it was $1.83 \pm 0.50‰$, and in the 9 rats sacrificed in the last 8 h of the experiment, it was $3.20 \pm 0.75‰$. This agrees with reports in the literature that the latent period of action of thyroid hormone on cell division may be 24-48 h [6] or even longer [5].

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