EFFECT OF PARAAMINOBENZOIC ACID ON CHOLESTEROL SYNTHESIS AND BREAKDOWN IN THE LIVER OF INTACT AND ATHEROSCLEROTIC RABBITS

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We have previously demonstrated [1] that novocain and its hydrolysis product paraaminobenzoic acid (PABA) inhibit the development of atherosclerosis in experimental animals. These results indicated that it would be valuable to investigate the effect of PABA on cholesterol metabolism in the liver by making direct determinations of the cholesterol content of weighed samples of minced liver incubated under various conditions.

EXPERIMENTAL METHOD

Samples of minced liver weighing 2.5-3.0 g were incubated at 37-38° in the presence of 5 ml of 5% sodium acetate solution or 5 ml of 1% potassium phosphate solution, stimulating the breakdown of cholesterol in the liver. The samples were oxygenated every hour and incubated for 3 h, after which they were hydrolyzed with alkali. Cholesterol was extracted from the hydrolyzate with small portions of ether, and determined by the ordinary calorimetric method using the Liebermann-Burchard method. Experiments were conducted on 32 rabbits.

EXPERIMENTAL RESULTS

The first series of experiments was carried out on intact animals. It will be clear from Table 1 that the initial cholestrol concentration in the liver of the intact rabbits averaged 223.8 ± 6.5 mg%. In samples of minced liver from the same animals but incubated with sodium acetate the cholesterol concentration was 12.7% higher, and in samples from the same animals incubated with potassium phosphate an increase in the concentration was observed in only 2 cases, while in the remaining three samples the cholesterol level was below its initial value, i.e., breakdown of cholesterol had taken place.

Some of the experiments of the first series were conducted on rabbits receiving preliminary injections of PABA subcutaneously in a dose of 10 mg/kg for a period of 2 weeks. The rabbits were then sacrificed and the liver extracted and investigated by the method described above. The results of experiments with these rabbits (Table 2) showed that

TABLE 1. Concentration of Cholesterol (in mg %) in the Liver of Intact Rabbits Incubated under Various Conditions

Rabbit No.	Initial concentration	Incubation with sodium acetate	Incubation of potassium phosphate	Synthesis (in %)	Breakdown (in %)
1	236	264,4	244,6	12	—
2	236	273	300	15,7	—
3	207	236	182	14,1	12,1
4	214	239	201	10	6,2
5	226	252	203	11,5	10,2
Mean . . .	223,8±6,5	252,8±7,8	226±23,5	12,6±1,3	9,5±0,9

TABLE 2. Cholesterol Concentration (in mg %) in the Liver of Rabbits Receiving PABA for 2 Weeks, Incubated in Various Conditions

Rabbit No.	Initial concentration	Incubation with sodium acetate	Incubation of potassium phosphate	Synthesis (in %)	Breakdown (in %)
6	384	283	361,3	Does not take place	6
7	346,6	346	306	» »	12
8	380	306	280	» »	27
9	270	265	215	» »	20,5
10	236	209	184	» »	22
Mean . . .	323,3±29,5	302±29	269±35,5		20,5±4,5

TABLE 3. Cholesterol Concentration (in mg %) in the Liver of Rabbits during Regression of Atherosclerosis

Rabbit No.	Initial concentration	Incubation with sodium acetate	Incubation of potassium phosphate	Synthesis (in %)	Breakdown (in %)
		Control			
11	700	647	700	Does not take place	
12	500	484	466	» »	
13	733	730	733	» »	
14	697	685	695	» »	
15	703	700	701	» »	
Mean . . .	666,6±47	692,2±17,5	695±18,5		
		After administration of PABA			
16	303	271	268	Does not take place	12
17	473	430	443	» »	7
18	420	420	403	» »	4
19	446	450	383	» »	18
20	416	413	380	» »	9
21	316	280	261	» »	17,5
22	321	278	263	» »	18,1
Mean . . .	385±24	363,1±34,6	343±31,6		12,2±2,3

the preliminary administration of PABA increases the breakdown of cholesterol in all the samples of minced liver incubated with potassium phosphate and suppresses the synthesis of cholesterol in samples incubated with sodium acetate.

In the second series of experiments the synthesis and breakdown of cholesterol in the liver were studied in 14 rabbits during regression of experimental atherosclerosis. These experiments lasted 8 months.

In a control group (7 rabbits), after administration of cholesterol (in a daily dose of 0.5 g/kg for 16 weeks) had been discontinued, the animals received daily subcutaneous injections of physiological saline throughout the experimental period. A slow fall in the serum cholesterol concentration to 737.5 ± 7 mg %, and in the phospholipids to 245.7 ± 27.8 mg %, was observed towards the end of the experiment. The cholesterol/phospholipids ratio was 3.0.

Atheromatosis of the aorta was severe in the control animals, and a particularly large accumulation of extensive, confluent atheromatous plaques was observed in the thoracic division of the aorta. The lipid concentration in the aortic tissue was 11.5 mg/100 mg. The initial level of the liver cholesterol was high, on the average 666.6 ± 47 mg % (Table 3). In addition, total suppression of biosynthesis of cholesterol from sodium acetate and of breakdown

of cholesterol in the samples containing potassium phosphate was observed in 100% of cases. The blocking of the biochemical processes in the liver (synthesis and breakdown) may apparently be attributed to the high initial concentration of cholesterol in the liver (666.6 mg %). Our findings agree with data in the literature [2].

In the experimental group, consisting of 7 rabbits, after administration of cholesterol had been discontinued the animals were given subcutaneous injections of PABA in a dose of 5 mg/kg twice daily for 16 weeks. Most of these rabbits revealed significant increases in the translucency of the blood serum from 30 to 35 days after the beginning of PABA administration, with corresponding falls in the cholesterol level from the initial values (from 1280 to 323 mg %). The phospholipid concentration in the serum was 380 mg %. At the end of the experiment the serum cholesterol and phospholipid levels in the experimental animals returned permanently to normal.

In most rabbits the degree of atheromatosis of the aorta was slight. Only small accumulations of lipids were found in the arch of the aorta and at the mouths of the great vessels. The lipid content of the aortic tissues was 5.5 ± 0.7 mg/100 mg. In the animals receiving PABA the initial cholesterol concentration in the liver was lower, averaging 385 ± 24 mg %, i.e., only 59% of the control value, and the breakdown of cholesterol in the samples with potassium phosphate was increased by 12.2% (Table 3) and synthesis of cholesterol in the samples of minced liver containing sodium acetate was totally suppressed.

Following these results, experiments were also carried out to study the effect of PABA on the synthesis and breakdown of cholesterol in the liver in vitro. Samples were taken from the liver of intact animals weighing 1.5 kg and kept on a normal diet. In 18 of the 20 experiments the breakdown of cholesterol was increased on the average by 24% over its initial value in the liver, except in two experiments in which it was unchanged.

It may be concluded from these experimental results that PABA influences the cholesterol metabolism in the liver. By stimulating the breakdown of cholesterol in the liver, PABA thereby apparently leads to the more rapid regression of experimental atherosclerosis in animals, as shown by a fall in the concentration of cholesterol, phospholipids, and total lipids in the aorta of the experimental animals.

SUMMARY

A study was made of the effect of paraaminobenzoic acid (PABA) on the synthesis and disintegration of cholesterol in the liver (V. N. Kolmakov's method) in intact rabbits and in those with developed atherosclerosis. The experiments demonstrated that PABA administered in a dose of 10 mg/kg depressed cholesterol synthesis and intensified its disintegration both in intact rabbits and in animals with developed atherosclerosis.

LITERATURE CITED

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