THE EFFECT OF NOVOCAIN AND CERTAIN NEW ANTICHOLINERGIC DRUGS ON THE COURSE OF EXPERIMENTAL ATHEROSCLEROSIS

A. F. Ryzhova

From the Department of Pharmacology (Head—Professor T. A. Mel'nikova)
of the Leningrad Institute of Pharmaceutical Chemistry
(Presented by AMN SSSR Active Member S. V. Anichkov)
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That regression of the atherosclerotic process is possible has been demonstrated both clinically and experimentally in studies of its pathogenesis and morphogenesis and of methods for its treatment and prevention [1, 4, 10]. This makes it very important to find new pharmacological substances which will prevent or arrest the development of atherosclerosis.

The substances which have been investigated in this connection include those inhibiting the central nervous system (Luminal, Barbamyl [sodium amytal], chloral hydrate, et al.), or inhibiting the development of lipoidosis in the arterial walls, drugs which stimulate the central nervous system (Phenamine [amphetamine sulfate], caffeine et al.), which act to intensify the alimentary hypercholesteremia of lipoidosis in the aorta, and substances affecting the autonomic nervous system [15 et al.].

The association of the atherosclerotic process with endrocine disturbances has prompted investigations of the effect of hormonal preparations on the course of atherosclerosis: Thyroidin [dessicated thyroid preparation] [14], synestrol and testosterone propionate [7], and adrenocorticotropic hormone [3].

This research has led to the discovery of several substances which arrest the development of atherosclerosis: β-sitosterol, certain vitamins, vanillone, phenexane, etc.

Works by Rumanian scientists [12] have shown the beneficial effect of Novocain on age changes in the blood vessels.

Moreover, N. E. Kavetskii [5] has shown that the administration of a combination of Novocain and ascorbic acid eliminates hypercholesteremia in patients with atherosclerosis.

A study of cholesterol metabolism during regression of experimental atherosclerosis in rabbits has demonstrated that Novocain helps reduce the cholesterol stably combined with proteins [8].

On the other hand, N. T. Kovaleva [6] concluded that the course of experimental atherosclerosis deteriorates under the influence of Novocain. This conclusion was based only on Novocain's effect on the blood cholesterol.

There are, therefore, contradictions in the literature data cited concerning Novocain's effect on the course of atherosclerosis. This led us to conduct an experimental study of Novocain's effect on the course of atherosclerosis compared with that of various anticholinergic agents.

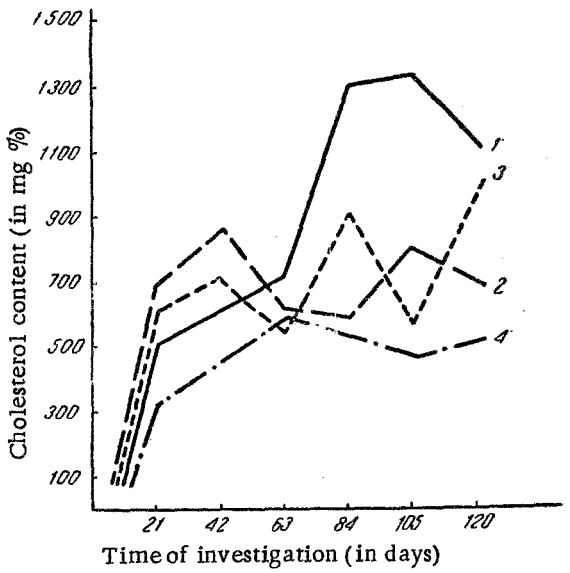
METHOD

In the first series of experiments, cholesterol was administered perorally through a gastric probe by N. N. Anichkov's generally accepted method in a dose of 0.5 g per rabbit daily in the form of a 10% solution in oil for 120 days. The second series of experiments was performed on animals in which atherosclerosis was induced by a daily feeding of 3 g cholesterol combined with 50 g grated carrot for a period of 90 days [16]. The course of the atherosclerotic process was controlled by the blood cholesterol level, as determined by the Grigo method, and the lecithin content as determined by the Fiske-Subbarow method (for phosphorus). The animals were sacrificed at different intervals after the experiment began. We studied the morphological changes in the animals' aortas and determined the total content of lipids by extracting them from the wall, using the method employed by K. G. Volkova. All the experimental substances were administered to the animals subcutaneously.

RESULTS

The experiments were performed on 27 rabbits, which were divided into five groups.

The first, or control, group of five animals was given cholesterol and a physiological solution daily. Pronounced cholesteremia was observed in the rabbits of this group during the cholesterol feeding period, reaching an average level of 1477 mg% ±187.33 toward the end of the fourth month (see figure).



Cholesteremia curves of control and experimental rabbits. 1) Control; 2) cholesterol + Novocain; 3) aprophene; 4) diprophene.

After four months of observation, the animals of the control and experimental groups were sacrificed by air embolism. The heart and all of the aorta above its bifurcation were removed. Macroscopically, we found the walls of the aortas in the three control rabbits to be thickened by the development of large, connected atheromatous plaques which projected into the vascular lumen. Plaques were observed along the whole length of the aorta, but were particularly marked in the thoracic section. Four plus marks (++++) were used to denote the degree of atheromatosis found in these rabbits; the atheromatosis found in the aorta of the other two control rabbits was somewhat milder. Lipid deposits were also observed in the thick mass of the aortic valves.

Microscopic examination of sections from the aorta, stained with sudan III, showed an irregular thickening of the intima by lipid deposits and atherosclerotic plaques in all five of the control rabbits.

The lipids extracted from the aortas of the control animals constituted an average 118 mg±11.7 which, with an average aorta weight of 825.4 mg±123.5, comes out to 14.3 mg±4.7 of lipids per 100 mg of tissue (Table 1).

In the second group (seven rabbits), to which Novocain (10 mg/kg) was administered, we observed rapidly increasing cholesteremia during the first six weeks of the experiment. The blood cholesterol level went up to 800 mg%, but was somewhat lower (600-700 mg%) by the end of the fourth month. The atherosclerotic changes in the aorta were considerably less pronounced than in the control (++ in three rabbits and + in two).

The average amount of lipids found in the aortas of the animals of this group was 35.6 mg±2.9, with an average aorta weight of 611.3 mg±63.1, so that 5.6 mg ±0.43 of lipids was found per 100 mg of aortic tissue (see Table 1).

Under the microscope, most of the preparations made from the rabbits' aortas showed small lipid deposits in the tunica interna; the tunica media of one rabbit also showed such deposits.

In the third group of rabbits (five animals), which was given cholesterol + aprophene [a,a-diphenylpropionic acid β-diethylaminoethyl ester hydrochloride] (5 mg/kg), we observed a gradual increase of blood cholesterol during the first nine weeks of the experiment (to 500-600 mg%) and a still greater increase in the cholesterol level toward the end of the experiment (see figure). In most of the animals, the morphological changes in the aorta approximated those in the control. A high degree of aortic atheromatosis (+++) was found in three rabbits, a somewhat milder degree (++) in two.

Microscopically, the aorta showed considerable lipoid deposits in the tunica interna and an accumulation of foam cells. Diffuse lipoid infiltration was found in separate sections of the tunica media.

The fourth group of rabbits (five animals) received diprophene [thiodiphenylacetic acid β-di-n-propylaminoethyl ester hydrochloride] (5 mg/kg). Throughout the experiment (120 days), the cholesterol level in all five rabbits was the lowest of all the experimental series, the maximum being 500-550 mg% (see figure).

The morphological changes in the aortas were negligible. The microscope preparations showed some thickening of the intima due to "pulverulent" lipoid deposits in the tunica media, in the form of "dust" in the foam cells.

The fifth group of animals (five rabbits) received aprophene (5 mg/kg) + Novocain (10 mg/kg). In these animals, the blood cholesterol level was high, approximating that in the experimental series with aprophene. The average amount of lipids extracted from the aortas was 73.3 mg±6.2, or 9.7 mg±0.81 per 100 mg aortic tissue, the average aorta weight being 747.8±51.2 (see Table 2).

TABLE 1. Content of Lipids (in mg%) in Aortic Wall of Rabbits (Average Data)

Substances administered	Average content of lipids	Weight of aorta (in mg)	Content of lipids per 100 mg aorta
Control	118	825.4	14.3
Novocain	36.6	611.3	5.6
Diprophene	68.2	879.7	8.5
Aprophene	93	765.8	12.1
Aprophene + Novocain	73.3	747	9.7

TABLE 2. Cholesterol and Lecithin Content in Blood of Rabbits Given Novocain and Diprophene during Regression of Atherosclerosis (Average Data)

Time investigated (from beginning of experiment)	Animals given				Control animals	
	novocain		diprophene			
	cholesterol	lecithin	cholesterol	lecithin	cholesterol	lecithin
14 days	817	352	1027	508	819	490
28 "	469	317	796	409	781	276
42 "	386	240	392	237	484	194
56 "	132	108	260	165	584	253

In a second series of experiments (15 rabbits), we studied the effect of Novocain and diprophene on atherosclerosis regression. The investigations were performed on three groups of rabbits with experimentally reproduced atherosclerosis.

In the control group (five rabbits), the blood cholesterol level at the end of the cholesterol feeding period (90 days) was 819 mg%±127.37; subsequently, it gradually decreased to 584 mg%±93.45 at the end of the experiment. A parallel decrease in the lecithin content was noted.

In all the rabbits of the control group, the aorta, particularly the upper part, was covered with large atheromatous plaques jutting out sharply into the lumen, except for one rabbit, in which less pronounced aortic changes were found.

The average lipoid content of the aortic wall was 12.7±3 mg per 100 mg aorta weight (see Table 2).

In the next group of rabbits (five animals), which were given Novocain at the end of the cholesterol feeding period, the blood cholesterol level decreased rapidly from 817 mg%±173.25 to 132 mg%±39.95 at the end of the experiment. The cholesterol level fell to the original norm in two animals. We also observed a decrease in the lecithin content, with a slight increase at the end of the experiment.

A moderate degree of aortic lipoidosis was found in the rabbits (++). The greater part of the surface of the aortic intima was free from atheromatous formations, no marked accumulation of lipids being found except in the arch; small, separate islets were observed along the whole inner surface of the aorta in only two rabbits.

The amount of lipids extracted from the aortas constituted an average of 4.7 mg±0.55 per unit of weight (Table 3).

In the last group (five rabbits) given diprophene, the blood cholesterol level regularly decreased from 1027 mg% at the start of the diprophene administration to 260 mg±71.5 at its end; a gradual decrease in the lecithin content was also observed.

Macroscopically, the aortas of this group exhibited only mild changes; most of the aortic wall was free from lipoid deposits. Some accumulation of the latter was observed only in the arch of the aorta and on its valves. The average amount of lipids extracted from the aorta was 56 mg±5.8, or 6.9 mg±1.15 per 100 mg aorta weight, the average aorta weight being 816.5 mg±109.

When the data of the first and second series of experiments are compared, it is evident that diprophene and, especially, Novocain inhibit the development of lipoid infiltration of the aorta and promote quicker absorption of lipids during the regression period. The data of our investigations coincide with those of T. K. Borozdina and D. I. Semenova [2]. We assume that the stimulating effect of Novocain and diprophene on the hypophysis–cortex–adrenal gland system is part of the mechanism of their beneficial effect on experimental atherosclerosis. In this connection,

N. V. Mikhailova [9] demonstrated intensified ACTH secretion attending Novocain administration, while A. N. Poskalenko [13] observed an analogous phenomenon under conditions of diprophene administration. Moreover, there are indications in the literature that the ACTH inhibits the development of cholesterol atherosclerosis [3].

TABLE 3. Content of Lipids (in mg%) in Aortic Wall after Treatment with Novocain and Diprophene

Control			Novocain			Diprophene		
wt. of aorta (in mg)	total lipoidosis		wt. of aorta (in mg)	total lipoidosis		wt. of aorta (in mg)	total lipoidosis	
	in whole aorta	in 100 mg aortic tissue		in whole aorta	in 100 mg aortic tissue		in whole aorta	in 100 mg aortic tissue
820	80	9.7	790	45.0	5.6	695	60.3	8.2
1300	108	8.3	620	50.0	8.0	1200	50.7	4.2
950	70	7.3	870	50.0	5.7	717	70.2	9.7
850	102	12.0	530	31.5	6.2	667	50.0	7.5
720	111	15.2	615	37.2	6.5	802	40.0	5.0
Average 928±111.2	94.2±9	12.7±3	685.6 ±69	42.7±5.7	4.7±0.56	817.4 ±109	56.6±5.8	6.9±1.15

Another reason for Novocain's beneficial effect could be its ability to reduce the stability of the bonds binding cholesterol to proteins.

Novocain's ability to enhance the general trophic process in the organism also seems to play a large part in this effect.

SUMMARY

Blood cholesterol levels were found to rise steadily with progressive development of experimental atherosclerosis attaining 1477 mg% ± 187.33 by the end of the fourth month in untreated rabbits (control). Administration of Novocain and of certain anticholinergic drugs (aprophene and diprophene) simultaneously with the cholesterol feeding resulted in lower blood cholesterol levels in all of the experimental animals as compared to controls; development of atherosclerosis, evaluated in terms of lipids deposited in the aorta, proved to have reached a lower degree in the experimental group.

During regression of atherosclerosis (after cholesterol administration had been discontinued), Novocain and diprophene rapidly brought about an abrupt reduction of the blood cholesterol level and hastened the removal of lipids from the aortal wall. Novocain was found to produce a more favorable effect as compared to the other drugs under consideration.

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