THE EFFECT OF LIPOCAIC ON THE CHOLINERGIC REACTION OF THE BLOOD IN DOGS UNDERGOING PARTIAL PANCREATECTOMY

I. N. Volkova and O. S. Kochnev

Department of Physiology (Head – Dr. Med. Sci. I. N. Volkova) of the Kazan Medical Institute

(Presented by Active Member AMN SSSR N. N. Zhukov-Verezhnikov)

Translated from Byulleten Éksperimental' noi Biologii i Meditsiny Vol. 49, No. 4, pp. 41-44, April, 1960

Original article submitted March 30, 1959

It has been shown by A. V. Kibyakov and his coworkers [3, 4] that partial extirpation of the pancreas leads to a temporary disturbance of acetylcholine synthesis in animals. The mechanism of this phenomenon has not received adequate study.

According to investigations by many workers, partial depancreatization causes no significant changes in the level of the blood sugar [2, 3, 12, 17], whereas the blood lipid concentration falls appreciably [16]. Total depancreatization is always accompanied by the development of fatty infiltration of the liver, which is not removed by insulin [13, 15], but which is prevented by the influence of the lipotropic factor of the pancreas [5, 13, 15]. On the basis of the many investigations by S. M. Leites and his coworkers [5, 7, 8], this active principle of the pancreas is its second hormone, lipocaic. This hormone activates oxidation of the fatty acids in the liver and the formation of phospholipids [6, 9, 10]. Phospholipid metabolism is associated with the synthesis of acetylcholine.

We conceived the idea that the cause of the disturbance of acetylcholine synthesis during partial depancreatization of animals is a deficiency of the hormone lipocaic. In order to verify this hypothesis we investigated the influence of lipocaic on the formation of acetylcholine in animals subjected to a preliminary partial pancreatectomy. The index selected of the acetylcholine metabolism was the blood level of acetylcholine in experimental animals and the serum cholinesterase activity.

METHOD

Experiments were carried out on dogs. From the saphenous vein, 5 ml of blood was drawn into a syringe containing 5 ml of eserine solution in a concentration of 1 : 50,000. The blood was defibrinated and diluted with Ringer's solution in a ratio of 1 : 10. The total dilution of the blood was thus 1 : 20. The acetylcholine concentration in the blood was estimated by Corsten's method [14] in the isolated frog's lung, in which contractions develop at an acetylcholine concentration of up to $1 \cdot 10^{-20}$. The exised lung of a frog was washed free from blood and kept in a solution of eserine (1 : 10,000) for three hours in the cold. The lung was then fixed in a small glass vessel (10 ml in volume), which was constantly perfused with Ringer's solution. The contractions of the lung were recorded on a kymograph. The blood for testing, and acetylcholine in different dilutions, was added to the fluid in the glass vessel in a volume of 1 ml. After each test the lung was thoroughly washed until complete relaxation was achieved. At the beginning and end of the experiment the degree of sensitivity of the lung to acetylcholine was tested. The acetylcholine concentration in the blood for testing was determined by means of the concentration of acetylcholine solution causing a contraction of the lung of equal height on the kymograph, with a subsequent calculation for whole blood.

The serum cholinesterase activity was estimated by a titration method, as described by T. V. Pravdich-Neminskaya [11]. The method is based on the decomposition of acetylcholine by the cholinesterase of the test serum with the formation of choline chloride and an equivalent quantity of acetic acid. The acetic acid was titrated with a 0.01 N solution of NaOH in the presence of a 0.02% solution of cresol red as an indicator. The titration was carried out from a microburette of 0.2 ml capacity and with divisions of 0.002 ml. The percentage of acetylcholine decomposed in unit time was taken as a measure of the activity of the enzyme.

The investigations described were carried out in the first place on unoperated dogs, and later at various periods after the operative removal of part (3/5) of the pancreas, with simultaneous ligation of its ducts. Daily observations were made on the animals until 30-38 days after operation. Of the six dogs used in the experiment, three were controls and did not receive lipocaic in the postoperative period; starting on the first day after the operation, the other three dogs received five tablets of lipocaic, i.e., 50 units, daily with their food. All the dogs were of roughly the same weight and were kept on a normal diet (meat, bread and meal). The experiments were performed at the same time of day. The animals were kept under the same conditions during the pre- and postoperative periods.

RESULTS

Before partial extirpation of the pancreas, the acetylcholine concentration in the blood of all the experimental dogs was $2 \cdot 10^{-6}$–$2 \cdot 10^{-4}$, and the serum cholinesterase

activity fluctuated between 29.6 and 19.1%. The blood acetylcholine level and the degree of cholinesterase activity on different days varied within very narrow limits in each animal.

After partial pancreatectomy a considerable fall in the blood acetylcholine level was observed in the three dogs which did not receive lipocaic. This fall was apparent after the second postoperative day and reached its maximum on the sixth day after operation. The concentration of acetylcholine in the blood was only $2 \cdot 10^{-15} - 2 \cdot 10^{-14}$. Later, the acetylcholine level began to rise, but on the 8th-14th day it was still low ($2 \cdot 10^{-12} - 2 \cdot 10^{-9}$ Even on the 38th day after operation the acetylcholine concentration in the blood had still not reached fully its preoperative level (acetylcholine was determined in the blood in concentrations of $2 \cdot 10^{-8} - 2 \cdot 10^{-7}$). At the same time as the fall in the acetylcholine concentration, a fall in the serum cholinesterase activity was also observed. The maximum fall was observed on the sixth postoperative day (3.6-9.1%) and was followed by a gradual rise, but on the 38th day after operation the cholinesterase activity had not reached its initial values. The serum cholinesterase activity varied between 15.4 and 18.3% at this period after operation (the 38th day).

In the three depancreatized dogs which received lipocaic in the postoperative period, the pattern of the changes in the cholinergic reaction of the blood was different. In the first days after operation a fall in the blood acetylcholine level was observed, and this was especially pronounced on the sixth day after operation; the degree of this fall, however, was much less than that observed in the control animals. The blood acetylcholine level was $2 \cdot 10^{-11} - 2 \cdot 10^{-5}$. In the subsequent postoperative days the blood acetylcholine concentration rose rapidly. Whereas on the eighth day after operation the blood acetylcholine level was still low ($2 \cdot 10^{-8} - 2 \cdot 10^{-5}$) by comparison with the normal values for these animals, on the 14th day after operation the blood acetylcholine had fully regained its initial values ($2 \cdot 10^{-4} - 2 \cdot 10^{-6}$) in two of the experimental dogs, and in only one dog was this restoration of the initial level delayed until the 18th day after operation. On all the following days of observation (until the 30th-38th postoperative day) the blood acetylcholine level was maintained within normal limits (preoperative period). The serum cholinesterase activity in these dogs on the 6th-8th day was slightly lowered (17.2-11%), and on the 14th day it had reached normal in one dog and nearly normal in the other two dogs. On the subsequent postoperative days the serum cholinesterase activity was identical with its value in the preoperative period. The course of the changes in the blood acetylcholine level and in the serum cholinesterase activity in the individual experimental dogs in the postoperative period is demonstrated by the figures in the table.

In the animals receiving the hormone lipocaic after partial pancreatectomy, the weakening of the cholinergic reaction of the blood was thus less prolonged and much less pronounced than in the control animals. In our experiments, side by side with the fall in the acetylcholine level, a fall in the serum cholinesterase activity was ob-

Animals	Concentration of acetylcholine in the blood					Serum cholinesterase activity (in %)				
	before operation	after operation				before operation	after operation			
		6th day	8th day	14th day	38th day		6th day	8th day	14th day	38th day
Dogs subjected to partial depancreatization										
Santa	$2 \cdot 10^{-5}$	$2 \cdot 10^{-15}$	$2 \cdot 10^{-11}$	$2 \cdot 10^{-9}$	$2 \cdot 10^{-7}$	21	3,6	10,9	15,4	15,4
Chernyi	$2 \cdot 10^{-5}$	—	$2 \cdot 10^{-10}$	$2 \cdot 10^{-10}$	$2 \cdot 10^{-8}$	20,9	—	11,0	18,1	18,3
Barbos	$2 \cdot 10^{-6}$	$2 \cdot 10^{-14}$	$2 \cdot 10^{-12}$	$2 \cdot 10^{-10}$	$2 \cdot 10^{-8}$	20,0	9,1	13,6	17,2	18,85
Dogs receiving lipocaic after depancreatization										
Lokhmatyi	$2 \cdot 10^{-4}$	$2 \cdot 10^{-5}$	$2 \cdot 10^{-5}$	$2 \cdot 10^{-4}$	—	29,6	17,2	16,8	26,0	—
Kerri	$2 \cdot 10^{-6}$	$2 \cdot 10^{-9}$	$2 \cdot 10^{-8}$	$2 \cdot 10^{-6}$	$2 \cdot 10^{-6}$	19,1	16,4	16,0	19,1	19,2
Ovra	$2 \cdot 10^{-6}$	$2 \cdot 10^{-11}$	$2 \cdot 10^{-8}$	$2 \cdot 10^{-8}$	$2 \cdot 10^{-6}$	21,0	11,0	13,6	16,4	20,2

Changes in the Concentration of Acetylcholine in the Blood and the Serum Cholinesterase Activity in Depancreatuzed Dogs not Receiving and Receiving Lipocaic

served. The cholinesterase present in the serum of dogs is known to be mainly nonspecific and its activity is maximal at relatively high concentrations of acetylcholine [1]. The decrease in the serum cholinesterase activity in the depancreatized dogs was thus, in itself, an index of the disturbance of acetylcholine formation. On the other hand, the increase in the serum cholinesterase activity in the depancreatized dogs receiving lipocaic was evidence of the normalization of the acetylcholine metabolism under the influence of this hormone.

SUMMARY

From analysis of the results obtained it may be concluded that lipocaic is able to a considerable degree to prevent those disturbances of acetylcholine formation which as a rule are found at certain postoperative periods in depancreatized animals. The main cause of these disturbances is consequently a deficiency of this hormone. This suggests that the pancreatic hormone, lipocaic, plays a part in the control of the synthesis of acetylcholine, one of the most important mediators of the nervous system. The results which we obtained are of obvious interest in the evaluation of the action of lipocaic in clinical con-

LITERATURE CITED

[1] D. E. Al'pern, Abstracts of Proceedings of the Eighth Congress of Physiologists, Biochemists, and Pharmacologists, [in Russian] (Moscow, 1955) p. 26.

[2] K. Z. Kan, Byull, Eksptl. Biol. i Med. 20, 4-5, 45 (1945).

[3] A. V. Kibyakov and A. A. Uzbekov, Byull. Eksptl. Biol. i Med. 3, 202 (1950).

[4] O. D. Kurmaev, Transactions of the All-Union Society of Physiologists, Biochemists and Pharmacologists 1, 85 [in Russian] (Moscow, 1952).

[5] S. M. Leites, Uspekhi Sovremennoi Biol. 22, 2 (5), 231 (1946).

[6] S. M. Leites and T. S. Yakusheva, Arkh. Patol. 11, 4, 44 (1949).

[7] S. M. Leites, Problemy Endokrinol. i Gormonoterap. 1, 1, 71 (1955).

[8] S. M. Leites and I. A. Eskin, Problemy Endokrinol. i Gormonoterap. 3, 5, 12 (1957).

[9] G. T. Pavlov, Byull. Eksptl. Biol. i Med. 25, 3, 244 (1948).

[10] G. P. Pavlov, Byull. Eksptl. i Med. 2, 126 (1952).

[11] T. V. Pravdich-Neminskaya, Doklady Akad. Nauk SSSR 65, 3, 405 (1949).

[12] F. M. Allen, J. Metabolism, Res 2, 219 (1922).

[13] F. N. Allan, D. J. Bowie, et al. Brit. J. Exper. Path. 5, 75 (1924).

[14] M. Corsten, Arch. ges. Physiol. 244, 281 (1940).

[15] L. R. Dragstedt, I. van Prohaska, and H. P. Harms, Am. J. Physiol; 117, 175 (1936).

[16] G. R. Hlavacek, P. V. Harper, et al. Am. J. Physiol. 179, 439 (1954).

[17] O. Minkowski, Arch. Exper. Path. und Pharmakol. 31, 85 (1893).