observations [8, 14]. One of the important physiological consequences of a fall in the diastolic blood filling of the left ventricle is the fact that under these circumstances the basic mechanism of compensation of cardiac activity (the Frank–Starling mechanism) is impeded. This may aggravate the disturbances of the hemodynamics and blood supply to the heart and may lead to progression of the myocardial lesions caused by cytotoxic damage to the heart.

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ATHEROSCLEROSIS INDUCED IN RABBITS BY PROLONGED ELECTRICAL STIMULATION OF THE HYPOTHALAMUS

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The effect of prolonged electrical stimulation of emotiogenic zones of the hypothalamus in rabbits on their blood lipid level and the development of atherosclerosis was studied with the aid of a specially designed autonomous system. A negative emotional state observed during stimulation was accompanied by hyperlipemia and by the development of atherosclerosis in one third of uncastrated and two thirds of castrated animals.

KEY WORDS: *Electrical stimulation of the hypothalamus; emotions; lipids; atherosclerosis.*

During prolonged emotional stress induced by frequent interchange of a stereotype of higher nervous activity animals develop atherosclerosis without the addition of cholesterol to the diet [7]. The writers have previously studied the basic mechanisms of its development [8-10].

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TABLE 1. Changes in Indices of Lipid Metabolism, Glucocorticoids, and PBI during Electrical Stimulation of Hypothalamus (M ± m)

Procedure	Time of investigation	β-Lipoproteins, mg%	Cholesterol, mg %	Triglycerides, mg%	Phospholipids, mg%	FFA, meq/liter	Glucocorticoids, μg%	PBI, μg%
Control	Before insertion of electrodes	232±11,2	28,8±0,9	96,4±10,5	71,0±5,6	0,26±0,03	6,2±0,5	5,0±0,32
	After operation:							
	1 month	260±10,5	26,0±1,2	75,8±5,9	72,6±3,2	0,28±0,02	6,3±0,02	5,6±0,40
	P	>0,05	>0,05	>0,05	>0,05	>0,05		>0,05
	3 months	268±13,0	29,4±1,5	83,6±9,1	74,2±4,8	0,35±0,05	7,9±0,8	4,8±0,30
Coagulation of ventromedial model	Before insertion of electrodes	254±10,4	29,1±2,9	98,6±6,9	81,1±6,6		7,8±0,6	4,5±0,27
	After coagulation:							
	1 month	216±9,9	33,4±1,4	78,4±5,5	79,0±6,1		6,9±0,6	
	P	<0,05	>0,05	>0,05	>0,05			
	3 months	294±10,2	34,4±1,5	113,3±12,0	93,3±9,4		7,3±0,5	5,9±0,35
Electrical stimulation	Before insertion of electrodes	231±5,3	39,1±2,2	74,1±2,8	76,2±2,8	0,36±0,03	6,5±0,4	4,8±0,29
	Stimulation:							
	1 month	274±10,8	61,6±3,3	132,6±6,0	90,5±3,8	0,75±0,04	10,9±0,8	7,6±0,72
	3 months	451±15,9	98,5±5,3	212,6±8,2	110,6±5,7	0,78±0,015	4,3±0,4	7,2±0,61
	P	<0,001	<0,001	<0,001	<0,001	<0,001	<0,01	<0,01
Electrical stimulation after castration (females)	Before insertion of electrodes	254,3±15,8	53,4±3,9	99,7±8,6	95,2±7,4	0,28±0,06	7,5±0,6	5,1±0,28
	Stimulation:							
	2 weeks	327±28,2	76,5±8.0	127,0±17,5	115,5±5,2	0,81±0,04	12,6±0,9	8,8±0,65
	P	<0,05	<0,02	>0,05	<0,05	<0,001	<0,001	
	3 months	694,4±92,4	121,8±17,8	256,6±45,9	135,8±14,6	0,52±0,03	4,1±0,5	6,8±0,35
	P	<0,001	<0,01	<0,01	<0,05	<0,001	<0,001	<0,001

In this investigation the effect of negative emotional stress, induced by prolonged electrical stimulation of the hypothalamus, on lipid metabolism, on the function of certain endocrine glands, and on the development of atherosclerosis was studied.

EXPERIMENTAL METHOD

Experiments were carried out on 69 rabbits of both sexes weighing 3-3.5 kg. Bipolar electrodes (nichrome, 100 μ) were inserted into the region of the ventromedial hypothalamus, into nuclei located around the third ventricle (ventro- and dorsomedial, periventricular), and into the dorsolateral and caudal portions of the posterior hypothalamus, known to be the centers of "fear" and "rage." The coordinates of the corresponding structures were calculated from Fifková and Marsala's atlas. The electrocorticogram was recorded through unipolar electrodes. Electrodes were inserted into the ten control rabbits but they were not subsequently stimulated. In five rabbits the region of the ventromedial nuclei was destroyed by coagulation (current 5 mA, exposure 5 sec). Prolonged electrical stimulation was given to 45 rabbits. Finally, nine rabbits (females) were stimulated after castration. A specially designed autonomous system (volleys of square pulses with a frequency of 100 Hz, pulse duration 0.5 msec, stimulus duration 0.1 sec, voltage 1 V, interval between stimuli 4.5 min), weighing 105 g, fixed to the rabbit's spine, which did not interfere with the animal's usual mode of life, was used.

Behavioral responses were studied in free-behavior chambers, and the EEG and autonomic responses in a chamber in which measured functional loads could be applied. Continuous stimulation of the hypothalamus for 30 days alternated with rest periods of 2 weeks. Every 10 days the blood levels of β-lipoproteins (turbidimetrically), cholesterol (by the Liebermann–Burchard method after extraction of lipids by Folch's method), triglycerides [15], phospholipids [13], and free fatty acids (FFA) [12] were determined. Adrenal and thyroid function was judged from the blood levels of glucocorticoids [4] and protein-bound iodine (PBI) [5]. At the end of the experiment the animals were killed. To determine the location of the electrodes the brain was fixed in 10% formalin and then embedded in paraffin wax. A series of frontal sections was stained with hematoxylin and eosin. The aorta was stained with Sudan III *in toto* and affected areas were investigated histologically for their lipid content (by staining with Sudan III and hematoxylin).

EXPERIMENTAL RESULTS AND DISCUSSION

During prolonged electrical stimulation of the hypothalamus changes developed in cortical and subcortical electrical activity of the brain, and a combination of behavioral and

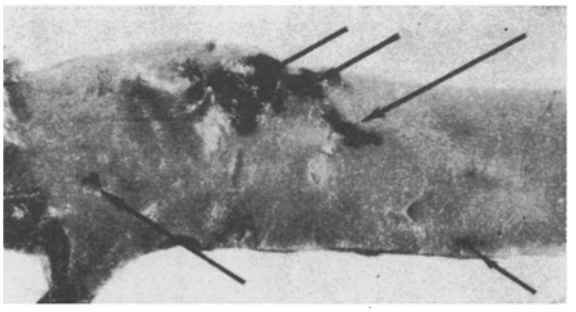

Fig. 1

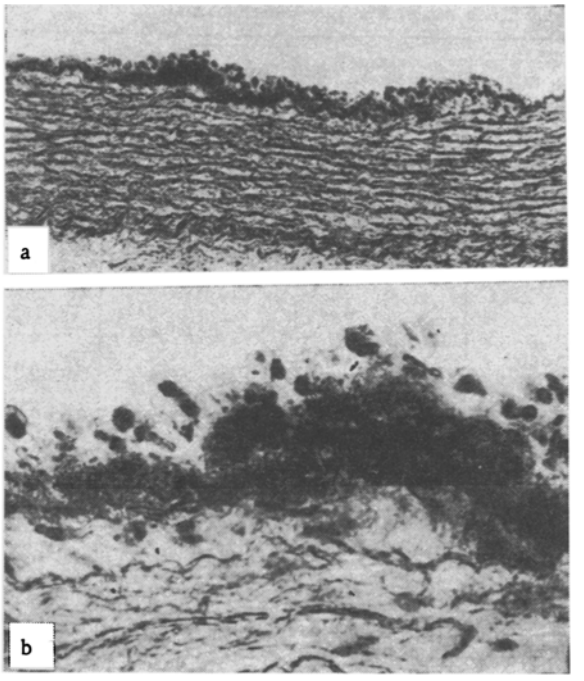

Fig. 2

Fig. 1. Specimen of arch and thoracic part of aorta of castrated rabbits after hypothalamic stimulation. Arrows indicate atherosclerotic plaques. Stained with Sudan III *in toto*.

Fig. 2. Deposition of lipids in intima of thoracic part of aorta of noncastrated rabbits after hypothalamic stimulation. Massive deposits of lipids can be seen in the intima and subintimal layer, where they are distributed in the ground substance and intracellularly. Sudan II and hematoxylin: a) 56×, b) 280×.

autonomic responses characteristic of negative emotional states developed. The animals' behavior in some cases was marked by passive fear and anxiety, whereas in other cases an aggressive defensive response was observed. As a rule these negative responses lasted throughout the period of observation.

Changes in lipid metabolism appeared after the first days of electrical stimulation. During prolonged stimulation the concentrations of cholesterol, triglycerides, and FFA rose progressively, and by the end of the first month they were 1.5 times higher than initially (Table 1). Hyperlipemia was combined with raised blood levels of glucocorticoids and PBI. Toward the end of the third month of stimulation the concentrations of β-lipoproteins and FFA were twice as high as initially, and those of cholesterol and triglycerides were 2.5 times higher, whereas the glucocorticoid concentration was lower. By itself, castration of the females caused a small increase in the β-lipoprotein and triglyceride levels. Castration has been shown [9] to lead to transient disturbances of the β-lipoprotein composition and to the formation of lipoproteins rich in triglycerides. As a result of hypothalamic stimulation the blood lipid fractions in the castrated rabbits rose more sharply and earlier than in animals with intact gonads. Considerable changes developed as early as after 2 weeks. Hyperlipemia in this period was combined with a high level of glucocorticoids and PBI. At the end of a 3-month period of stimulation the concentrations of β-lipoproteins, cholesterol, and triglycerides were 230-273% of their initial levels. In some experiments the β-lipoprotein level reached 1000 mg %, cholesterol 223 mg %, and triglycerides 509 mg %. Thyroid function remained a little increased, whereas the glucocorticoid level fell by 54% from its initial value.

In the control rabbits all the indices studied showed no significant change throughout the experiments.

Histological examination of the aorta showed deposits of lipids in animals exposed to hypothalamic stimulation (Figs. 1 and 2). The lipids were deposited in the intima and subintimal layer, in the ground substance, and intracellularly. Single thrombi could be observed.

In 36 of the 45 rabbits receiving hypothalamic stimulation the electrodes were located in the region of the ventromedial, dorsomedial, and periventricular nuclei, and also in nuclei of the caudal part of the posterior hypothalamus. In 16 of these 36 rabbits atherosclerotic lesions were found in the aorta. In castrated females the electrodes were located in the region of the ventromedial nucleus and the dorsolateral region. Atherosclerosis of the initial and abdominal parts of the aorta was found in six of nine rabbits of this group.

Comparison of the location of the electrodes with the behavioral responses shows that stimulation of the ventromedial and dorsolateral regions and the nuclei of the posterior hypothalamus was accompanied by the appearance of negative emotional states of fear or aggression, in agreement with observations by other workers [1, 3, 6].

An increase in certain blood indices of lipid metabolism (cholesterol, triglycerides) for a short time during brief stimulation of the hypothalamus and other structures of the limbic system has been described previously [2, 11, 14]. In the present investigation prolonged stimulation of emotiogenic zones of the hypothalamus, alternating with periods of rest, induced not only lasting changes in lipid metabolism, but also the development of atherosclerosis of the aorta.

The blood glucocorticoid concentration fell during prolonged electrical stimulation. Considering the results of previous investigations [9] deposition of lipids in blood vessels can be considered to take place during a period of insufficiency of the adrenal glucocorticoid function.

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