It can be concluded from these results that changes in the physical properties of the lipid matrix of nerve cell membrances induced *in vivo* and *in vitro* by LPO may have a significant effect on receptor–ligand interaction. The importance of the facts discovered is determined by the fact that endogenous LPO products may accumulate *in vivo* as a result of various physiological influences and also during the development of different forms of pathological states.

LITERATURE CITED

1. Yu. A. Vladimirov and G. E. Dobretsov, in: Fluoresent Probes in the Study of Biological Membranes [in Russian], Moscow (1980), p. 257.
2. V. E. Kagan, L. L. Prilipko, V. M. Savov, et al., Biokhimiya, No. 3, 482 (1979).
3. F. Z. Meerson, V. E. Kagan, L. L. Prilipko, et al., Byull. Éksp. Biol. Med., No.10, 404 (1979).
4. J. P. Bennet and S. H. Snyder, Mol. Pharmacol., 12, 373 (1976).
5. I. E. Dumont, Trends Pharmacol. Sci., 1, 219 (1980).
6. C. E. Dunlap, F. M. Leslie, M. Rado, et al., Mol. Pharmacol., 16, 105 (1979).
7. F. Hirata, W. J. Strittmatter, and J. Axelrod, Proc. Natl. Acad. Sci. USA, 76, 368 (1979).
8. M. Shinitzky and Y. Barenholz, Biochim. Biophys. Acta, 515, 367 (1978).

TYPES OF TRIGLYCERIDASES IN THE PIG AORTA

G. I. Yusupova

UDC 577.153.2:591.413

KEY WORDS: lipoprotein lipase; triglyceridase; aorta.

The triglyceridase activity of aortic tissue of different animals and man has been determined frequently [3, 7, 9, 10, 11]. It has usually been suggested that at least some of this activity is due to lipoprotein lipase. Nevertheless, data on the presence of lipoprotein lipase in the aorta are inadequate, and reliable evidence in support of this view has been obtained only in extracts of bovine aortas [1, 2].

Since the obtaining information on this topic is important, it was decided to study types of triglyceridases in the pig aorta.

EXPERIMENTAL METHOD

Pig aortas were obtained from an abattoir immediately after slaughter of the animals. All subsequent procedures were carried out at 4°C. Connective tissue and fat were removed from the aorta, which was washed with water and then cut into pieces with scissors. To 5 g of the tissue treated in this way 20 ml of 0.5M Tris-HCl, pH 8.0, was added, and the mixture was homogenized for 1 min in a Politron homogenizer. After extraction for 15-20 m in the homogenate was centrifuged for 20 min at 15,000g, and the cytoplasmic fraction was filtered through several layers of gauze.

The substrate emulsion for determination of triglyceridase activity was prepared by the method described in [8]. For this purpose 0.88 mg of glycerol-3-[1-^{14}C]-oleate with 0.1 ml of a 10% solution of ovolecithin was dispersed in 5.3 ml glycerol. The components of the substrate mixture were homogenized in a glass homogenizer.

To determine triglyceridase activity 0.1 ml of substrate was added to 0.1 ml of enzyme solution and incubated at 37°C for 1-3 h. Each sample contained 2.65 μg triolein, 3.3 $\times$ 10^5 cpm of glycerol-3-[1-^{14}C]-oleate (49 mCi/mmole), 0.06 mg ovolecithin, and 0.05 mg bovine serum albumin in 0.2 M Tris-HCl, pH 8.0. Fatty acids liberated during the reaction were extracted with a methanol–chloroform–heptane (1.41:1.25:1) mixture. Radioactivity was measured in a Mark 2 (Nuclear Chicago) liquid scintillation counter. When the effect of NaCl, protamine sulfate, or serum apoproteins on triglyceridase activity was studied, samples without the addition of these substances served as the control. Protein in the aortic extract was determined by Lowry's method [7]. The fraction of total apoproteins of the high-density lipoproteins was isolated from human blood serum by the method described in [4].

Laboratory of Physical Chemistry, Institute of Biological and Medical Chemistry, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Orekhovich.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 11, pp. 554-555, November, 1981. Original article submitted April 1, 1981.

0007-4888/81/9211-1508$07.50

TABLE 1. Effect of NaCl, Protamine Sulfate, and Heparin on Triglyceridase Activity

NaCl, M	Inhibition, %	Protamine sulfate, mg/ml	Inhibition, %	Heparin, units/ml	Inhibition, %
0,2	42,8	3,0	10	0,1	7
0,5	69,2	7,5	32	1,0	22
				10,0	34
1,0	79,0	15,0	60	100,0	91

The following reagents were used: triolein from Serva, glycerol-3-[1-^{14}C]-oleate from Amersham, heparin and protamine sulfate from Spofa, Tris from Fluka, bovine serum albumin from Biomed, and α-L-lecitin from the Olaine Chemical Reagents Factory.

EXPERIMENTAL RESULTS

Maximal lipolytic activity of the aortic extracts under the conditions used was observed at pH 8.0. Liberation of fatty acids from the triolein emulsion was linear, at least for 1-5 h at 37°C; the degree of hydrolysis of the triglyceride did not exceed 3%. Triglyceridase activity was 0.08 nmole fatty acid/h/mg protein of aortic extract. Triglyceridase activity from bovine aorta, determined with triolein dispersed ultrasonically in the presence of detergent, was 0.9 unit [1]. Variability of the data on specific triglyceridase activity in the aortas of animals of different species must be noted. One explanation of this fact may be the different conditions of processing the tissue and the different methods of obtaining the substrates (lipolytic activity is largely dependent on the physical state of the substrate). It is therefore difficult to compare results obtained under different experimental conditions.

The presence of lipoprotein lipase in the aortic extract was demonstrated by making use of its characteristic features such as inhibition of activity by NaCl and protamine sulfate, and activation by proteins of serum lipoproteins (apoprotein C-II of lipoproteins is known to activate lipoprotein lipase).

Data on the action of NaCl on the triglyceridase activity of the extract are given in Table 1. They show that NaCl inhibited lipolysis strongly. When NaCl was added in a concentration of 1M, only 20% of the initial triglyceridase activity could be detected.

The residual activity when inhibition of bovine aortic triglyceridase by 1M NaCl was studied [2] was 14%.

Table 1 shows that protamine sulfate, in a concentration of 3 mg/ml, depressed activity only very little, and the addition of increasing concentrations of protamine sulfate to the incubation medium led to substantial inhibition of lipolytic activity. Strong inhibition of activity was likewise observed when the action of protamine sulfate on bovine aortic triglyceridase was studied [2].

On the addition of total fraction of apoproteins of high-density lipoproteins (0.5-5 mg/ml) a 15-20% increase in triglyceridase activity was observed. The lipoprotein lipase of aortic extract was evidently already partially activated *in vivo*, and that was why a higher degree of activation was not obtained.

Stimulation of activity by low concentrations and inhibition by high concentrations of heparin was found with lipoprotein lipase from rat adipose tissue [5]. It was therefore interesting to determine the effect of heparin on the triglyceridase activity of aortic extract. As Table 1 shows, heparin (1-100 i.u./ml) depressed triglyceridase activity. It can be concluded from these results that both triglyceridase and lipoprotein lipase activities are heparin-sensitive.

It can thus be concluded from the data showing marked inhibition of triglyceridase activity of the pig aorta by NaCl and protamine sulfate and also its activation by apoproteins of high-density lipoporteins that lipoprotein lipase is present in this aorta. The quantity of this enzyme evidently corresponds to 60-80% of total trigylceridase activity.

LITERATURE CITED

1. C. W. M. Adams, O. B. Bayliss, Y. H. Abdulla, et al., J. Atheroscler. Res., 9, 87 (1969).
2. P. E. Dicorleto and D. B. Zilversmit, Proc. Soc. Exp. Biol. (New York), 148, 1101 (1975).
3. A. Dury, J. Gerontol., 16, 114 (1961).
4. R. S. Elkeles, Hormon. Metab. Res., 6, 151 (1974).
5. K. Hayase and B. F. Miller, J. Lipid Res., 11, 209 (1968).
6. L. C. Henson and M. C. Schotz, Biochim. Biophys. Acta, 409, 360 (1975).
7. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
8. P. Nilsson-Ehle, J. Lipid Res., 17, 536 (1976).
9. J. Patelski, Enzyme, 12, 299 (1971).

10. J. Patelski, D. E. Bowyer, A. N. Howard, et al., J. Atheroscler. Res., 8, 221 (1968).
11. B. Shore and V. Shore, Biochemistry (Washington), 8, 4510 (1969).

BLOOD CORTICOSTERONE LEVEL IN KRUSHINSKII-MOLODKINA AND WISTAR RATS AFTER SHORT EXPOSURE TO ACOUSTIC STIMULATION

M. M. Nikitina and L. G. Romanova

UDC 612.453.018:612.129:577.175.53/.014.45

KEY WORDS: acoustic stimulation; epileptiform fit; corticosterone; stress.

The effects of acoustic stimulation on rodents have been widely studied by many specialists. The epileptogenic action of sound has received the most attention. Lines of mice and rats particularly sensitive to acoustic stimulation and responding to it by epileptiform fits have been bred [1]. One such line is the Krushinskii-Molodkina (KM) line. Rats of this line respond to short acoustic stimulation by a strong clonicotonic fit. Long exposure to acoustic stimulation leads to severe functional disturbances and often to death of the animal. The high sensitivity of KM rats to sound suggests that this stimulus acts as a stress factor for these animals. Systemic protective-adaptive processes, largely controlled by hormones of the pituitary-adrenal system, are known to be a very important component in the development of stress reactions [7].

In the investigation described below the functional state of the adrenals was investigated in rats exposed to short acoustic stimulation.

EXPERIMENTAL METHOD

Male KM rats, which in 98-99% of cases reacted to acoustic stimulation by clonicotonic fits, and Wistar rats, insensitive to sound, were used in the experiments.

The experimental animals were exposed to the loud ringing of a bell (about 110-115 dB) for 1.5 min. The sensitivity of the rats to sound was assessed on a four-point scale [1].

The state of adrenal function was studied by determining changes in the blood corticosterone level of the rats in response to acoustic stimulation. Blood was taken from the animals by decapitation 5, 15, 30, 60, and 120 min after acoustic stimulation. Intact animals, not exposed to acoustic stimulation, were used to determine the initial blood hormone level.

The corticosterone concentration in the blood plasma was determined by competitive protein-binding analysis using cortisol-1,2-H^3 with rat serum transcortin as binding protein [6]. Different types of stress have been characterized previously on the basis of information on the blood glucocorticoid concentrations of experimental animals: stress associated with immobilization, laparatomy, laser irradiation, and during the formation of the structure of the society [2-4]. This method of assessing the state of function of the pituitary-adrenal system is highly sensitive and it adequately reflects the level of the protective-adaptive reactions in states of stress.

EXPERIMENTAL RESULTS

The plasma corticosterone level of intact Wistar rats did not differ significantly from that of intact KM rats (Table 1). As a result of exposure for 1.5 min to acoustic stimulation 13% of the Wistar rats developed motor excitation and a fit assessed at 2 points on average, actually during exposure to the stimulus. The remaining 87% of Wistar rats were absolutely insensitive to sound (0 point). The KM rats reacted in 99% of cases to the acoustic stimulus with a powerful epileptiform fit assessed at 4 points.

The blood corticosterone concentration of Wistar rats insensitive to sound was increased 30 min after exposure, but 1 h after acoustic stimulation it returned to its initial level (Table 1). The increase in the blood corticosterone concentration in rats insensitive to sound suggests that acoustic stimulation evokes a short-term stressor effect in these animals.

Biological Faculty, M. V. Lomonosov Moscow University. (Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 11, pp. 555-557, November, 1981. Original article submitted June 19, 1981.

 0007-4888/81/9211-1510$07.50