EFFECT OF ADRENALIN EXCITATION ON BRAIN PHOSPHOLIPID AND THROMBOPLASTIN LEVELS

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As reflected in the content of total phospholipids (neutral and acid) and the degree of change in thromboplastic activity (TA) as a result of incubation of brain tissue homogenates from normal animals and from animals treated with adrenalin, the brain regions studied can be arranged in the following order: medulla > cerebellum > cerebral cortex. The effects of adrenalin are characterized by a decrease in TA, i.e., by changes opposite to those observed in TA under normal conditions. The role of phospholipids in the formation of the various factors of the clotting and anticlotting systems of the blood is discussed.

KEY WORDS: *brain phospholipids; adrenalin; thromboplastic activity.*

The mechanism of the stimulant effect of hyperglycemic states on blood clotting has so far received little study. This relationship is established to some degree through certain stages of phospholipid metabolism directly concerned with the formation of the various components of the blood clotting system and undergoing marked activation in states of excitation, especially due to adrenalin. Carbohydrates participate in lipogenesis through acetate [3, 9, 12], which is condensed into fatty acids, and certain products of glycolysis (phosphotrioses), which are converted into L-α-glycerophosphate. Among the phospholipids (PL) active in relation to blood clotting, the phosphatidylethanolamines are noteworthy: they are regarded [1, 2, 7] as structural components of thromboplastins, the biosynthesis of which takes place in many tissues and systems of the body, notably the blood cells, endothelium and, in particular, the brain.

This paper describes the results of investigations to study the dynamics of the various groups of PL — neutral (NPL) and acid (APL) — and also thromboplastic activity (TA) in homogenates of various parts of the rabbit brain incubated (in the presence of glucose) under normal conditions and in the presence of adrenalin.

EXPERIMENTAL METHOD

Homogenates of the medulla, cerebellum, cerebral cortex, and whole brain were incubated for 2 h at 37°C in medium of the following composition (in mM): NaCl 0.5, KCl 0.5, $MgSO_4$ 0.66, and glucose 7.0. The homogenates were obtained in Tris-HCl buffer and added to the incubation mixture in a dose of 200 mg fresh tissue. A solution of adrenalin (Sigma, USA) was injected intraperitoneally into rabbits in a single dose of 0.05 mg/kg, and the animals were killed 1 h later. PL were fractionated by chromatography on paper with silica gel [11]. Glucose was determined by the method of

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TABLE 1. Dynamics of TA (prothrombin time, in sec), Content of Total and Individual PL (in μg lipid phosphorus/g wet weight of tissue) in Homogenates of Various Parts of Rabbit Brain and Total Brain Homogenate under Normal Conditions and after Treatment with Adrenalin (M ± m)

Parameter studied	Medulla			Cerebellum			Cerebral hemispheres			Total brain homogenate		
	normal			normal			normal			normal		
	before incubation	incubation	adrenalin incubation	before incubation	incubation	adrenalin incubation	before incubation	incubation	adrenalin incubation	before incubation	incubation	adrenalin incubation
Total PL	1902,0 ± 24,0	2410,0±23,0 $P<0,001$	1401,0±20,5 $P<0,001$	1872,0 ± 26,7	2223,0±18,7 $P<0,001$	1271,0±14,5 $P<0,001$	1522,0 ± 16,1	1946±17,7 $P<0,001$	1266,0±29,6 $P<0,001$	1737,0 ± 18,8	2280,0±17,0 $P<0,001$	1361,0±23,4 $P<0,001$
Total NPL (SM + L + EP)	1410,0 ± 29,5	1880,0±13,5 $P<0,001$	992,0±15,3 $P<0,001$	1368,0 ± 22,5	1766,0±16,9 $P<0,001$	1945,0±29,2 $P>0,001$	1006,0 ± 23,1	1424,0±25,8 $P<0,001$	959,0±10,7 $P<0,001$	1260,0 ± 12,7	1740,0±18,3 $P<0,002$	1011,0±18,8 $P<0,001$
Total APL (MP + SP + PGP)	492,0 ± 24,8	530,0±17,0 $P=0,05$	409,0±18,0 $P<0,001$	504,0 ± 17,1	457,0±14,3 $P<0,002$	326,0±12,0 $P<0,001$	516,0 ± 17,8	522,0±18,1 $P=0,01$	307,0±12,0 $P<0,001$	477,0 ± 14,0	540,0±16,2 $P=0,002$	350,0±15,6 $P<0,001$
NPL/APL	3,0 ± 0,42	3,5±0,28 $P=0,05$	2,4 ± 0,25 $P<0,002$	2,6 ± 0,3	4,0 ± 0,25 $P<0,001$	2,9 ± 0,26 $P=0,002$	1,9 ± 0,3	2,7 ± 0,25 $P<0,002$	3,1 ± 0,3 $P<0,05$	2,6 ± 0,22	3,3 ± 0,32 $P<0,01$	2,8 ± 0,3 $P<0,1$
TA	18,3 ± 0,3	16,4±0,7 $P=0,02$	20,7 ± 0,2 $P<0,001$	19,0 ± 0,4	17,5 ± 0,5 $P<0,01$	21,1 ± 0,8 $P<0,001$	21,9 ± 0,4	20,2 ± 0,5 $P<0,001$	23,4 ± 0,3 $P<0,002$	20,7 ± 0,7	19,2 ± 0,7 $P=0,02$	23,2 ± 0,8 $P<0,002$

Note: SP) sphingomyelins; L) lecithins; EP) ethanolamine-phospholipids; MP) monophosphoinositides; SP) serine-phospholipids; PGP) polyglycerophospholipids.

Hagedorn and Jense, TA (as prothrombin time, in sec) in the homogenates by Quick's method in Kudryashov's modification [8].

EXPERIMENTAL RESULTS

The results (Table 1) show that incubation of brain homogenates of normal animals in buffered salt medium in the presence of glucose leads to an increase in total PL mainly on account of NPL. The NPL/APL ratio in homogenates of the medulla, cerebellum, cerebral cortex, and whole brain of the normal animals was 3.0, 2.6, 1.9, and 2.6 respectively, rising after incubation to 3.5, 4.0, 2.7, and 3.3. Intraperitoneal injection of adrenalin was followed by a decrease in the PL content in the homogenate (mainly on account of NPL) and by lengthening of the thromboplastin time to 20.7, 21.1, 23.4, and 23.2 sec respectively. These results are in agreement with the effect of adrenalin (as estimated previously on the basis of the arteriovenous difference) in the form of an increase in the PL content in the blood flowing from the dog's brain, a decrease in the content of these compounds in brain tissue, and activation of lipolytic reactions catalyzed by phospholipases; this is particularly true of phospholipase D, which splits PL-glycerides, including phosphatidylethanolamines, with the liberation of free ethanolamine and an increase in the blood level of this compound [5], which is known to be a powerful stimulator of biological reactions and, in particular, of blood clotting.

Adrenalin excitation was accompanied by marked activation of blood clotting, during which the free thromboplastin level was lowered both in the blood and in the brain tissue [5]. In all probability this resulted from intensive utilization of thromboplastin in the reaction of thrombin formation, with a consequent increase in the blood thrombin level which led to a high degree of blood clotting.

The decrease in the content of NPL and TA in the incubated brain homogenates of rabbits receiving adrenalin evidently can be attributed both to the transport of these compounds and to their partial hydrolysis. The results show that, as reflected in the levels of total PL, NPL, and TA and the value of the NPL/APL ratio, the parts of the brain studied can be arranged in the following order: medulla > cerebellum > cerebral cortex. The effects of adrenalin were manifested as diametrically opposite changes in the dynamics of TA and in the content of NPL and APL.

These observations point to the important role of the constancy factor in quantitative relationships between the various PL groups in maintenance of the normal background TA. Disturbance of this

constancy leads to appropriate shifts of TA and, consequently, to disturbances of the clotting system of the blood as a whole. In particular, these results confirm the concept put forward by Kreps [6] to explain the role of PL in CNS function.

It is difficult to accept a direct role of glucose and adrenalin in blood clotting. In whatever concentrations used, they had no activity in accelerating or retarding blood clotting, as is the case when many known factors of the blood clotting system are used. The results point to an important indirect action of these compounds on the state of blood clotting.

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