TIME COURSE OF AMMONIA RELEASE AND BINDING AND OF ACID PROTEINASE AND GLYCOLYTIC ENZYME ACTIVITY DURING EXPERIMENTAL TOXICO-ADRENAL ENCEPHALOPATHY

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Consecutive single injections of Escherichia coli endotoxin and adrenalin into dogs have been shown to cause a degenerative disease of the brain which, without any additional external influences, may follow a progressive course [3]. However, the endogenous mechanisms of development of this pathological process are unknown.

The aim of this investigation was to study the state of the metabolic system for release and binding of ammonia, and activity of acid proteinase and enzymes of energy metabolism in the cerebral cortex of dogs in the course of experimental toxico-adrenal encephalopathy (ETAE).

EXPERIMENTAL METHOD

Experiments were carried out on 155 mature mongrel dogs of both sexes weighing 8-20 kg. The control group consisted of 25 dogs. There were three series of experiments. In series I (30 dogs) E. coli strain 821 endotoxin (prepared in the Bacteriologic Laboratory, Central Research Institute of Hematology and Blood Transfusion), in a dilution of 1:10,000 and in a dose of 10 μg/kg, was injected into a subcutaneous limb vein of the animals. Animals of groups II (30 dogs) and III (95 dogs) were fixed to a frame before the experiment and the hair of the neck was shaved. Under local anesthesia with 0.25% procaine solution a piece of skin was excised from the neck. The common carotid artery was dissected from the vagus nerve, exteriorized, and sutured to the skin flap. After the operation wound of the dogs of series II had healed, a 0.1% solution of adrenalin in a dose of 75 μg/kg was injected in the course of 10-15 sec into the common carotid artery. The dogs of series III received an injection of E. coli endotoxin (10 μg/kg) into a subcutaneous limb vein, and 30-40 min later, adrenalin (75 μg/kg) was injected into the common carotid artery. Under pentobarbital anesthesia (40 mg/kg) the dogs were decapitated in series I and II 30 min, and 1 and 15 days, and in series III 30 min and 1, 15, 30, and 60 days after the injections. To determine concentrations of ammonia, glutamine, glutamate, aspartate, and GABA and glutamine synthetase activity the brain was removed in the course of 10-25 sec and placed in liquid oxygen. To determine activity of acid proteinase, hexokinase, phosphohexoisomerase, and glucose-6-phosphate dehydrogenase (G6PDH) the brain was quickly transferred into ice-cold physiological saline. The cortex was isolated. Ammonia was determined by the microdiffusion method [12] in the modification in [7], glutamine by the method in [4], and glutamate, aspartate, and GABA by electrophoresis on paper [8]. Glutamine synthetase activity was determined by the method [9] in the modification in [10, 13], and acid proteinase activity by the method [6], using hemoglobin as the substrate. Hexokinase activity was investigated by the method [5], phosphohexoisomerase by the method [1], and G6PDH by the method [2].

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TABLE 1. Effect of Endotoxin on Metabolic Parameters of the Dog Cerebral Cortex (M ± m)

Parameter	Control	Time after injection		
		30 min	1 day	15 days
Ammonia	0,38±0,09	2,01±0,16*	1,76±0,25*	0,50±0,27
Glutamine	5,40±0,08	13,12±0,42*	10,02±0,56*	6,20±0,82
Glutamine synthetase	40,0±1,19	60,0±3,60*	61,0±3,30*	39,0±2,00
Glutamate	9,20±0,50	6,40±0,48*	5,60±0,50*	10,00±0,82
Aspartate	3,12±0,06	2,40±0,16*	3,27±0,30	3,40±0,24
GABA	2,27±0,09	2,05±0,21	2,30±0,15	2,42±0,30
Acid proteinase	3,00±0,18	5,10±0,33*	6,40±0,46*	3,80±0,56
Hexokinase	10,82±0,85	8,85±0,66*	7,96±0,35*	9,76±0,96
Phosphohexoisomerase	9,78±0,35	7,12±0,38*	6,24±0,25	8,88±0,56
G6PDH	20,23±0,98	25,52±1,54*	24,64±0,86*	19,14±0,74

Legend. Here and in Tables 2 and 3 ammonia concentration expressed in μmoles amino nitrogen/g wet weight of tissue; glutamine concentration in μmoles amido nitrogen/g wet weight of tissue; glutamine synthetase activity in mg glutamylhydroxamic acid synthesized by 10 g of acetone powder in 30 min; glutamate, aspartate, and GABA concentrations in μmoles/g wet weight of tissue; acid proteinase activity in μg tyrosine/g protein; hexokinase activity in mg glucose/g wet weight of tissue; phosphohexoisomerase activity in μmoles fructose/g wet weight of tissue; and G6PDH activity in μmoles NADP/g wet weight of tissue. *$p < 0.05$ compared with control. Number of investigations in control was 13, and 5 in each series of experiments.

TABLE 2. Effect of Adrenalin on Metabolic Parameters of the Dog Cerebral Cortex (M ± m)

Parameter	Control	Time after injection		
		30 min	1 day	15 days
Ammonia	0,38±0,09	0,90±0,07*	0,42±0,10	0,48±0,12
Glutamine	5,40±0,08	4,08±0,16	3,70±0,48*	6,10±0,36
Glutamine synthetase	40,0±1,19	41,6±2,70	45,6±4,00	38,0±2,80
Glutamate	9,20±0,50	9,80±0,54	10,20±0,67	8,80±0,37
Aspartate	3,12±0,06	3,12±0,10	2,98±0,25	2,97±0,30
GABA	2,27±0,09	1,26±0,15*	1,28±0,12*	2,80±0,50
Acid proteinase	3,00±0,18	3,90±0,72	2,70±0,15	3,50±0,30
Hexokinase	10,82±0,85	9,66±0,36	12,63±0,74*	11,01±0,76
Phosphohexoisomerase	9,78±0,35	8,70±0,74	11,56±0,80*	8,64±0,36
G6PDH	20,23±0,98	5,35±0,53*	10,43±0,64*	21,00±0,94

EXPERIMENTAL RESULTS

The increase in the ammonia concentration in the dogs' cerebral cortex induced by injection of endotoxin (Table 1) was combined with activation of ammonia removal. This was shown by an increase in glutamine synthetase activity and in the glutamine concentration and a decrease in the concentrations of glutamic and aspartic acids, which were evidently utilized as ammonia acceptors. The brief rise of acid proteinase activity was probably a response of the body to injection of endotoxin. Changes were found in the activity of enzymes of carbohydrate metabolism: inhibition of glycolytic enzymes (hexokinase and phosphohexoisomerase) associated with activation of G6PDH suggests that glucose was predominantly utilized as an energy-yielding substrate by the compensatory "endoergic" pathways [11]. Isolated injection of adrenalin caused a smaller increase in the ammonia concentration (Table 2). Concentrations of glutamine and of dicarboxylic acids and glutamine synthetase activity were unchanged. The relations between the enzymes of energy metabolism indicated that glucose utilization mainly followed the pathway of glycolysis. Thus isolated injection of the endotoxin and of adrenalin induced a combination of metabolic changes of compensatory and adaptive character. By the 15th the parameters tested had returned to normal.

In ETAE induced by combined injections of endotoxin and adrenalin, the disturbances of brain metabolism were different in principle and were aggravated with the course of time (Table 3). At all times of investigation coordination between the formation and binding of ammonia was disturbed, as shown by accumulation of large quantities of ammonia in the brain

TABLE 3. Metabolic Parameters of Dog Cerebral Cortex in the Course of ETAE (M ± m)

Parameter	Control (13)	Time after injection				
		30 min	1 day	15 days	30 days	60 days
Ammonia	0,38±0,09	3,05±0,39* (8)	3,65±0,31* (8)	3,24±0,29* (8)	3,28±0,17* (8)	2,90±0,35* (8)
Glutamine	5,40±0,08	12,23±0,51* (8)	5,21±1,04 (8)	12,73±0,85 (8)	9,39±0,93 (8)	11,33±0,95* (8)
Glutamine synthetase	40,0±1,19	28,5±3,90* (8)	39,3±3,02 (8)	42,4±2,66 (8)	34,5±3,04 (8)	26,4±2,43* (8)
Glutamate	10,79±0,09	3,60±0,19* (8)	5,62±0,47* (8)	7,88±0,23* (8)	3,04±0,11* (8)	4,60±0,22* (8)
Aspartate	3,12±0,06	1,19±0,06* (8)	2,19±0,19* (8)	2,93±0,25 (8)	1,93±0,11* (8)	1,40±0,08* (8)
GABA	2,27±0,09	1,34±0,23* (8)	1,03±0,18* (8)	3,33±0,20* (8)	0,74±0,10* (8)	1,43±0,15* (8)
Acid proteinase	3,00±0,18	4,50±0,21* (11)	3,70±0,30 (11)	5,30±0,32* (11)	6,90±0,42* (11)	7,40±0,41* (11)
Hexokinase	10,82±0,85	7,15±0,29* (11)	9,77±0,54 (11)	7,25±0,48* (11)	9,55±0,57 (11)	5,23±0,41* (11)
Phosphohexoisomerase	9,78±0,35	9,06±0,50 (11)	5,07±0,20* (11)	5,43±0,29* (11)	4,75±0,54* (11)	7,86±0,22* (11)
G6PDH	20,23±0,98	13,10±0,56 (11)	16,45±0,52 (11)	5,67±0,34* (11)	39,60±3,13* (11)	15,06±0,24* (11)

Legend. Number of experiments given in parentheses.

tissues and the absence of any compensatory activation of glutamine synthetase. Under these conditions a high glutamine level can probably be explained by inhibition of glutaminases, and the low concentration of dicarboxylic acids and of GABA by insufficiency of the tricarboxylic acid cycle and also by their utilization as tissue respiration substrates, the insufficiency of which was shown by inhibition of activity of glycolytic enzymes. In the late stages of the course of ETAE the disturbances of brain metabolism were complicated by a lasting increase in acid proteinase activity.

Thus by contrast with the disturbances of brain metabolism induced by isolated injection of endotoxin and of adrenalin, the disturbances in ETAE are characterized by a lasting imbalance between the metabolic systems, manifested by lack of coordination between the processes of ammonia release and binding, inhibition of the enzymes of energy metabolism, and activation of acid proteinase. The neurochemical changes found correlate with neurohistologic features of the metabolic disturbances observed at the same times during the course of ETAE [3], and they may accordingly be regarded as one of the conditions for endogenous development of the pathological process.

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