is normally in a stable state and blockade by naloxone can be clearly revealed if the system is first activated by a suitable stimulus.

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NEUROCHEMICAL MECHANISMS OF THE ACTION OF EUPHYLLINE IN CEREBROVASCULAR INSUFFICIENCY

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In cerebrovascular insufficiency the ammonia concentration in the brain tissue shows a marked increase, glutamate dehydrogenase (GD) activity was reduced, and there is no significant change in the levels of glutamine and protein amino groups. Euphylline, which promotes normalization of the disturbed cerebral circulation, removes the excess of ammonia by restoring GD activity in the reductive amination reaction. The effect of euphylline is most clearly demonstrated in the presence of acute cerebral ischemia, evidence of the effect of the drug on the neurochemical mechanisms of compensatory regulation of the cerebral blood flow.

KEY WORDS: Euphylline; cerebral circulation; nitrogen metabolism; glutamate dehydrogenase.

A leading role in the therapeutic effect of euphylline in cerebrovascular disturbances is ascribed to the part played by the vascular component in its action [1, 10, 12]. The writers established the ammonia-neutralizing action of euphylline [2, 6], which is in harmony with views regarding the role of neurochemical mechanisms in compensatory regulation of the cerebral blood flow [4, 5]. This was the basis for the study of the effect of euphylline on the system of ammonia formation and removal under conditions of a reduced blood flow.

METHODS

Experiments were carried out on noninbred adult rats. The dynamics of changes in the indices of nitrogen metabolism and in the blood supply to the rats' brain was studied after unilateral occlusion of the common carotid artery. The local cortical blood flow was deter-

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TABLE 1. Effect of Euphylline on Concentration of Ammonia, Glutamine, and Protein Amino Groups and Total GD Activity in the Brain after Unilateral Occlusion of Common Carotid Artery in Rats (M ± m)

Index	Experimental conditions		
	control	occlusion of carotid artery	euphylline, 24 mg/kg intraperitoneally
Ammonia	0,59±0,02 (35)	1,2±0,04* (17)	0,62±0,01* (15)
Amino nitrogen of glutamine	3,0±0,14 (15)	3,1±0,15 (15)	3,3±0,13 (15)
Labile protein amino groups	19,8±0,21 (10)	19,4±0,1 (10)	19,5±0,16 (10)
Firmly bound protein amino groups	44,9±0,21 (10)	44,4±0,14 (10)	44,6±0,13 (10)
GD activity, reductive amination	0,33±0,01 (8)	0,22±0,02* (8)	0,35±0,04* (8)
GD activity, oxidative deamination	0,34±0,03 (8)	0,32±0,03 (8)	0,36±0,05 (8)

Legend. Content of ammonia, amino nitrogen of glutamine, and protein amino groups expressed in mg %, GD activity in μmoles pyridine nucleotide/mg protein/min; number of experiments shown in parentheses; statistically significant changes denoted by asterisk.

mined quantitatively by the hydrogen clearance method [9] in the modification in [3]. Ammonia and amino nitrogen of glutamine were determined by a microdiffusion method [8], and protein amino groups by the yield of ammonia after hydrolysis in 2 M H_2SO_4. Glutamate dehydrogenase (GD) activity was determined by a spectrophotometric method and expressed in Wroblewski units [7, 13]. Protein was determined by Lowry's method [11].

RESULTS

The results indicate that 24 h after unilateral ligation of the common carotid artery the concentration of free ammonia in the rat brain is considerably (more than twice) increased (Table 1). Intraperitoneal injection of euphylline under these conditions led to a decrease in the excess ammonia almost down to the control level; this action of the drug can be regarded as protective and restorative. In the light of these results it was necessary to study the effect of euphylline on mechanisms regulating the ammonia level in nerve tissue and to discover if the effect of euphylline was connected with removal of the excess ammonia or with prevention of its accumulation.

In the modern view, the formation and removal of ammonia is a cyclic process of degradation and resynthesis of nitrogenous compounds; the mechanisms regulating the ammonia level in nerve tissue are considered to be based on the following enzymic processes: amination and deamination of proteins, glutamine synthetase, glutaminase, glutamate dehydrogenase, and the adenylate system.

As the data given above show, in cerebral ischemia the glutamine amino nitrogen level in the brain was lowered by 20% compared with the control. Under the influence of euphylline the glutamine concentration was increased, but not significantly ($P > 0.05$), evidence that only a small part of the ammonia is involved in glutamine synthesis and that, consequently, the degree of participation of glutamine synthetase in the ammonia-removing effects of euphylline is negligible. At the same time it was shown that euphylline does not exert its effect through amination of the protein fraction of the brain, for the changes in the degree of amination of the brain proteins caused by euphylline were not significant.

It is clear that the increase in the excess of ammonia during cerebrovascular insufficiency was directly connected with inhibition of ammonia utilization associated with changes in GD activity.

Besides its role in amino acid biosynthesis and mechanisms of intracellular regulation of energy metabolism, GD also takes part in processes directed towards removal of ammonia in tissue. The results of the present experiments show that there was no change in the total GD

activity in oxidative deamination reactions during cerebrovascular insufficiency, whereas GD activity in the reductive amination reaction was reduced by 42%. Consequently, the increase in the excess of ammonia in cerebral ischemia can be explained by inhibition of one of the most important mechanisms of its removal, namely reductive amination of α-ketoglutarate. During administration of euphylline, total GD activity in the reductive amination reaction was significantly increased by 59%, almost up to the control values; consequently, an important role in the ammonia-removing effect of euphylline is played by its ability to restore GD activity. This characteristic action of euphylline is brought about essentially by its intervention in neurochemical processes responsible for maintaining the functional activity of the nervous system. Meanwhile the stabilizing action of euphylline is of great importance in the functioning of the intracellular mechanisms supplying energy to nerve tissue, which are closely linked with the physiologically normal activity of the brain.

In experiments in which the cerebral blood flow was recorded quantitatively the effect of euphylline on the blood supply to the brain under conditions of circulatory cerebral ischemia was studied. After unilateral occulsion of the common carotid artery in rats, as might be expected, there is a marked decrease in the local blood flow: One h after ligation the blood flow, which in the control was 45.5 ± 3.3 ml/min/100 g, was reduced by 41.7%, down to 27.0 ± 2.4 ml/min/100 g on the side of ligation ($P < 0.05$). Injection of euphylline under these conditions was accompanied by an increase in the local blood flow by 42.2%, and 15 min after injection of the drug the blood flow reached 38.6 ± 3.3 ml/min/100 g tissue ($P < 0.05$). Under conditions of cerebrovascular insufficiency, therefore, besides its stabilizing action on the leading neurochemical components, euphylline has the ability to restore the disturbed cerebral blood flow.

It should be noted that during prolonged cerebral ischemia (over 24 h after ligation), the effect of euphylline on the cerebral blood flow was very small; in 18% of the cases the blood flow in fact was not increased, but the effect was diametrically opposite — the local blood flow was reduced by euphylline on the side of occlusion. The reason for this effect of the drug must be that the effect of euphylline, in the presence of cerebrovascular insufficiency, is evidently determined predominantly by the neurochemical component of its action, whereas the vascular effects are realized later. Clearly in prolonged ischemia, accompanied by marked inhibition of neurochemical and also of vascular regulatory mechanisms, the effect of euphylline is exhibited only weakly, and may sometimes even be reversed.

These new aspects of the action of euphylline revealed by this investigation are thus evidence of its positive effect on the blood supply to the brain, depending on the severity of the brain lesion and the time of administration of the drug; they confirm its efficacy in the early stages of development of cerebrovascular insufficiency.

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