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ACTION OF ASCORBIC ACID ON BINDING OF ^{3}H-GABA AND ^{3}H-GLUTAMIC ACID TO CEREBRAL CORTICAL SYNAPTOSOMES IN RATS

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Ascorbic acid (AA) is contained in high and stable concentrations in the mammalian CNS [13]. We know that AA is a coenzyme of dopamine-β-hydroxylase, the enzyme which synthesizes noradrenalin [2] and which also possibly participates in dopamine synthesis [11]. However, concentrations of AA and catecholamines in brain structures do not correlate with each other [10] and AA evidently performs other, as yet unidentified, functions.

Reports of synaptic release of AA [9] and of changes in the firing rate of striatal neurons under the influence of AA [3] suggested that this compound is involved in synaptic transmission. With this suggestion, and also our own observations of the effect of intraventricular injections of AA on Na^+-dependent binding of ^{3}H-GABA [1], in mind it was decided to study the action of AA in vitro on binding of ^{3}H-GABA and ^{3}H-glutamic acid to cerebral cortical synaptosomes. The choice of these mediators was determined by the fact that GABA and glutamic acid are recognized neurotransmitters of cortical neurons [8], and the AA concentration in the neocortex is one of the highest to be found among brain structures [10]. In this investigation Na^+-depending binding, which is effected mainly by presynaptic receptors [15], was studied.

EXPERIMENTAL METHODS

The mitochondrial (synaptosomal) P_2 fraction was obtained from the cerebral cortex of decapitated male Wistar rats. Aliquots of this fraction, each containing 0.4-0.6 mg protein, in 0.1 ml of 0.32 M sucrose solution, were preincubated for 10 min at 0-4°C in 1.9 ml of Krebs–Ringer solution with or without L-AA (10^{-6}-10^{-3} M), after which ^{3}H-GABA (specific radioactivity 1.3 TBq/mmole, from Izotop, Leningrad) or ^{3}H-DL-glutamic acid (specific radioactivity 1.2 TBq/mmole, from Izotop) in a concentration of 10^{-7} M was added. Incubation was stopped after 10 min by centrifugation at 16,000 g and 4°C for 10 min. After washing twice with 50 mM Tris-HCl buffer (pH 7.4) the residue was dissolved in 0.1% Triton X-100 solution and the level of radioactivity was measured in Tritosol [4] on an Isocap-300 scintillation counter (Beckman, USA). Nonspecific binding was determined in the presence of 10^{-3} M unlabeled GABA or DL-glutamic acid; specific binding was calculated as the difference between total and nonspecific. The protein concentration was measured by Lowry's method [7].

EXPERIMENTAL RESULTS

The AA concentration in the incubation medium had a significant effect on Na^+-dependent binding of ^{3}H-GABA (Table 1). With low AA concentrations (from 10^{-6} to 10^{-5} M) specific binding of ^{3}H-GABA was doubled. A further increase in the AA concentration to 10^{-3} M steadily

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TABLE 1. Effect of AA on Specific and Nonspecific Binding of ^{3}H-GABA (in cpm × 10^3/min/mg protein) to Synaptosomes (M ± m)

AA concentration, M		Binding	
		specific	nonspecific
0	(8)	327,0±111,0	16,3±5,2
10^{-6}	(4)	238,0±53,8	14,9±3,1
10^{-5}	(4)	654,7±145,3**	25,2±3,0*
10^{-4}	(4)	410,2±58,7	16,6±4,2
$3\cdot10^{-4}$	(3)	341,5±61,5	18,7±3,0
10^{-3}	(4)	139,3±29,9**	7,0±3,2*

Legend. *P < 0.05, **P < 0.01 compared with results in absence of AA. Number of experiments given in parentheses.

TABLE 2. Effect of AA on Specific and Nonspecific Binding of ^{3}H-DL-Glutamic Acid (in cpm × 10^3/mg protein) to Synaptosomes

AA concentration, M	Binding	
	specific	nonspecific
0	131,2±34,1	20,5±8,4
10^{-5}	75,2±22,7*	12,5±1,1
10^{-4}	81,5±19,9*	9,6±1,6
10^{-3}	97,6±22,8	19,0±8,8

Legend. *P < 0.05 compared with results in absence of AA. Averaged results of four experiments given.

reduced mediator binding. Changes of a similar character under the influence of AA were found for nonspecific binding of ^{3}H-GABA.

Unlike ^{3}H-GABA, specific binding of ^{3}H-DL-glutamic acid by cerebral cortical synaptosomes was reduced only by increasing concentrations of AA(Table 2). The same tendency also was observed for nonspecific binding of ^{3}H-glutamic acid.

According to data in the literature, the AA concentration in the intercellular fluid of nerve tissue is about $3\cdot10^{-4}$ M [12]. As our observations show, this concentration does not affect binding of ^{3}H-GABA (Table 1), but an increase in its concentration in the incubation medium significantly reduced, and a decrease increased Na^+-dependent binding of ^{3}H-GABA. Binding of ^{3}H-DL-glutamic acid was increased somewhat as a result of an increase in the AA concentration. The opposite nature of the changes in Na^+-dependent binding of inhibitory (GABA) and excitatory (glutamic acid) mediators in response to a fall in the AA concentration may indicate the opposite character of changes in the GABA-ergic and glutamatergic systems of the neocortex when the extracellular ascorbate concentration is reduced in vivo also. In other words, a fall in the AA concentration in the intercellular space of the cerebral cortex may disturb the balance between inhibitory and excitatory influences in the neocortex. This emphasizes the special importance of homeostasis of AA, which is strictly maintained in the nervous system [12].

The results are evidence of the action of AA on presynaptic GABA and glutamic acid receptors. The effect of AA on binding of labeled ligands to α-adrenergic, dopamine, serotonin, muscarine, and opiate receptors was demonstrated previously [6, 14]. The action of AA on mediator receptors under these circumstances can be expected by the fact that it causes peroxidation of cell membrane lipids [15]. Evidently AA can induce peroxidation of lipids, which are components of all cell receptors and nonsepcific binding sites of mediators; changes in nonspecific binding of ^{3}H-GABA and ^{3}H-glutamic acid observed in the present experiments can be explained in the same way. Since the targets for the action of AA are membrane lipids, this will enable differences in the molecular structure of the receptors to be discovered. For instance, in the overwhelming majority of previous investigations the workers concerned

noted only the inhibitory action of AA on bonding of ligands to receptors. In the present investigation a twofold increase in Na^+-dependent binding of ^3H-GABA was observed when the AA concentration was 10^{-5} M. The results suggest that the presynaptic GABA receptor differs from monoamine, muscarine, glutamate, and opiate receptors in the accessibility of their lipid component to the peroxidative action of AA which, evidently, is linked with the specific features of the lipid composition and stereochemical structure of the presynaptic GABA receptor.

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