BIOPHYSICS AND BIOCHEMISTRY

Dextrorphan-Binding Proteins in the Hippocampus of Audiosensitized Rats Genetically Predisposed to Epilepsy

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A fraction of dextrorphan-binding proteins is isolated from the hippocampus of normal and audiosensitized Krushinsky—Molodkina rats genetically predisposed to epilepsy. Pharmacological analysis of this fraction reveals the presence of haloperidol-sensitive phencyclidine- (N-[1-Phenylcyclohexyl]piperidine, PCP)-binding proteins with molecular weight 18-20 kD judging from the data of high-performance liquid chromatography.

Key Words: *NMDA-glutamate receptor; σ-binding proteins; epilepsy*

At present, molecular and cell mechanisms of epilepsy attract great attention [7]. It is hypothesized that NMDA-glutamate receptors, together with calcium and potassium redistribution systems, play an important role in the pathogenesis of epilepsy [10]. Of particular clinical importance is the fact that antagonists of NMDA-glutamate receptors exhibit a pronounced anticonvulsive and antiepileptic effects [8]. Dextromethorphan and its o-demethylated derivative dextrorphan (DP) are of particular value, since they have no side psychomimetic effects in therapeutic doses and are well tolerable [8].

Pharmacological studies showed that apart from NMDA-glutamate receptors, DP with high affinity binds to σ-opiate receptors [6]. It is hypothesized that ligand-binding centers of σ-opiate receptors are pharmacologically similar to binding sites for noncompetitive inhibitors of NMDA-glutamate receptor [5]. Therefore, σ-receptor proteins are probably tightly coupled to NMDA-glutamate receptor and modulate its activity. In order to elucidate the mechanisms of interaction between σ-binding proteins with NMDA-glutamate receptor and to study molecular targets for DP, we purified and compared protein composition in the hippocampus of normal rats and rats predisposed to audiogenic epilepsy.

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MATERIALS AND METHODS

Experiments were carried out on male Wistar rats and rats of Krushinsky—Molodkina strain (KM) genetically predisposed to audiogenic epilepsy (body weight 200-250 g) [1].

Synaptic membranes were isolated from the hippocampus as described previously [2]. DP-binding proteins were solubilized with 1% sodium desoxycholate and centrifuged at 100,000*g* for 60 min. The supernatant was dialyzed against 20 mM Tris-HCl (pH 7.4) and applied to a DP-sepharose column (1×5 cm). Bound proteins were eluted with 10 μM DP, dialyzed, concentrated, and used in further analysis. DP-sepharose was prepared as described elsewhere [4].

High-performance liquid chromatography (HPLC) was carried out in a Waters system using a Protein

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Pak 300 SW column. Mobile phase contained 0.05 M phosphate buffer and 0.03% 3-[(3-Cholamidopropyl]dimethylammonio)-1-propanesulfonate (CHAPS), pH 6.8. The elution rate was 1 ml/min. Radioactivity (in 0.01-ml aliquot) and extinction (210 nm) of the eluate were determined.

Binding of radiolabeled ligands with proteins was measured in a medium containing 20 mM Tris-HCl, 2 mM EDTA, 5 μg/ml pepstatin, 5 U/ml aprotinin (pH 7.4), 30-50 μg/ml purified protein, and 5 nM ^{3}H-SKP 10047 (N-allylnormetazocine, 41.8 Ci/mmol, NEN) or ^{3}H-PCP (47.6 Ci/mmol, NEN). Nonspecific binding was measured in the presence of 10 μM DP or 10 μM haloperidol or 10 μM MK-801. Samples were incubated for 30 min at 25°C and filtered through Whatman GF/C filters or separated by HPLC.

RESULTS

The content of DP-binding proteins in the hippocampus of KM rats was higher than that in Wistar rats. After solubilization of the synaptic membrane proteins and purification on DP-sepharose, the preparations from KM rats contained more Lowry-positive material (by 1.3-1.5-fold) than those from Wistar rats (4.7 *vs.* 3.2 μg/g wet tissue). Moreover, the bulk of the DP-binding material isolated from the hippocampus of Wistar rats interacted predominantly with ^{3}H-SKP 10047 and weakly bound ^{3}H-PCP (Table 1). Haloperidol-sensitive sites in these proteins constituted 62-63%. ^{3}H-SKP 10047-binding proteins were sensitive only to MK-801, they weakly reacted with DP and did not bind haloperidol.

Thus, pharmacological analysis of DP-binding proteins revealed the presence of both NMDA-glutamate receptors interacting with PCP and MK-801 and σ-opiate receptors sensitive to haloperidol. Therefore, it can be concluded that DP interacts with both receptor systems.

The contents of ^{3}H-SKP 10047- and ^{3}H-PCP-binding proteins were practically the same in audiosensitized and normal rats. In both rat strains, ^{3}H-SKP 10047-binding proteins contained the same number of haloperidol-sensitive sites (62-63%). Unlike Wistar rats, the hippocampus of KM rats contained a higher number of PCP-binding sites with the same specificity for haloperidol and DP. The content of MK-801-binding proteins, and, consequently, the number of NMDA-glutamate receptors were similar in KM and Wistar rats (Table 1). Since PCP-binding proteins in KM rats are sensitive to both DP and haloperidol (σ-site), it can be concluded that NMDA-receptors in these rats possess an additional σ-specific regulatory site. This site presumably is not related to the major NMDA-receptor molecule, since the number of MK-801-binding sites is the same in KM and Wistar rats.

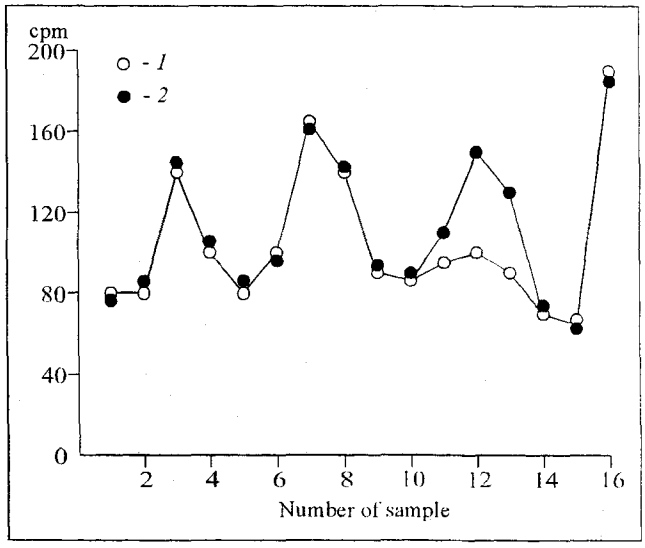

Fig. 1. High-performance liquid chromatography (HPLC) of dextrorphan-binding proteins on Protein Pak 300 SW. Radioactivity of HPLC fraction of hippocampal proteins incubated in the presence of ^{3}H-SKP 10047 from Wistar (*1*) and KM (*2*) rats.

For identification of the modulating sites, the DP-binding proteins were analyzed by HPLC. The HPLC spectrum of ^{3}H-SKP 10047-binding proteins was similar in both animal strains (Fig. 1). There were three peaks corresponding to molecular weights of 120, 60, and 15-20 kD. The contents of the first two fractions were the same in both strains, while the content of the low-molecular-weight fraction (15-20 kD) in KM rats considerably surpassed that in Wistar rats. These data suggest that the low-molecular-weight fraction contains haloperidol-sensitive proteins, which modulate the activity of NMDA-glutamate receptor in audiosensitive KM rats.

TABLE 1. Binding of Different Radiolabeled Ligands to DP-Binding Proteins from the Hippocampus of Wistar and KM Rats Solubilized and Purified from the Synaptic Membranes (*M±m*)

Animal strain	Specific binding, pmol/mg proteins	
	^{3}H-SKP 10047	^{3}H-PCP
Wistar (*n*=5)		
DP-specific binding	9.25±1.8	0.25±0.05
Haloperidol-specific binding	5.90±0.9	—
MK-801 specific binding	—	3.10±0.8
KM (*n*=7)		
DP-specific binding	7.65±1.2	1.55±0.29
Haloperidol-specific binding	4.65±0.7	1.75±0.3
MK-801 specific binding	—	3.12±0.85

Recent studies showed that DP is a promising anticonvulsant with a weak psychomimetic activity [10] It is hypothesized that similarly to other (+)-benzomorphans, the therapeutic effect of this drug is mediated through so-called σ-opiate receptor, which is not sensitive to naloxone but can be blocked by the neuroleptic haloperidol [6]. However, apart from the haloperidol-sensitive σ-receptors, (+)-benzomorphans interact with NMDA-glutamate receptors and modulate the binding of phencyclidine and MK-801 [5]. In case of MK-801 it is unknown whether these agents directly interact with channel part of the receptor, of their effect is mediated through σ-opiate proteins. Our experiments demonstrated the presence of additional low-molecular-weight proteins in the hippocampus of KM rats, which directly interact with NMDA-glutamate receptor and modulate its activity. This interaction possibly underlies the neuroprotective and anticonvulsant effects of (+)-benzomorphans.

Molecular weight of true σ-opiate receptor and mechanisms of signal transduction coupled with this receptor remain unknown [6]. It has been shown that molecular weight of DP-binding proteins greatly varies in different types of nervous cells (from 18 to 65 kD). Moreover, previous investigation haloperidol and other σ-active agents inhibit glutamate- and glycine-induced binding of ^{3}H-TCP [1-(2-thienyl) cyclohexyl piperidine] [9], and D-serine-stimulated accumulation of cGMP [3]. This implies that σ-site-bearing proteins can be a constituent of active NMDA receptor. Our findings suggest possible involvement of low-molecular-weight proteins in the modulation of glutamate receptors. These proteins probably modulate the sensitivity of NMDA-glutamate receptor and thereby contribute to the formation of an epileptic focus.

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