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Brain D2-Like Dopamine Receptor Distribution in Rats with Different Types of Genetic Epilepsy

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Abstract—The distribution of the D2-like dopamine receptor (D2DR) in the cortex and striatum was compared between rats with absence, audiogenic, or combined genetically determined epilepsy and normal Wistar rats by autoradiography. A significantly lower D2DR binding density was observed in the dorsal and ventrolateral aspects of the nucleus accumbens in epileptic vs. non-epileptic rats. Rats with audiogenic epilepsy additionally showed a higher D2DR density in the dorsal striatum and motor and somatosensory cortex and a lower D2DR density in the ventrolateral part of the nucleus accumbens. The findings indicated that a common neuronal circuit is involved in the pathogenesis of both convulsive and nonconvulsive forms of generalized epilepsy.

Keywords: absence epilepsy, audiogenic epilepsy, dopamine, neurotransmitter, autoradiography, WAG/Rij, KM
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INTRODUCTION

Absence epilepsy is a nonconvulsive general form of epilepsy and is determined by dyfunction of thalamocortical networks. The WAG/Rij inbred rat strain is genetically predisposed to absence epilepsy and provides its valid model [1]. The Krushinsky–Molodkina (KM) inbred strain is characterized by audiogenic epilepsy; KM rats display generalized motor seizures of a maximum intensity in response to auditory stimulation [2]. Part of the WAG/Rij population also demonstrate audiogenic seizures (WAG/Rij-AGS). This subpopulation provides a model of combined convulsive-nonconvulsive epilepsy. Functional insufficiency of the dopaminergic system is known for absence epilepsy [3]. Lower activity of the dopaminergic system is similarly observed in rats with genetic audiogenic epilepsy. The dopamine receptor distribution in the brain has previously been compared between WAG/Rij and ACI strains (the latter is resistant to epileptic disorders) by autoradiography. WAG/Rij have shown a higher D2-like dopamine receptor (D2DR) density in the parietal and frontal cortex and a lower density in the dorsal striatum and the hippocampus [4]. There is no data on the dopamine receptor distribution in rats with convulsive or combined epilepsy. The objective of this study was to compare the D2DR density distribution in Wistar rats and the three rat strains with genetically determined epilepsy.

MATERIALS AND METHODS

We used male rats of the Wistar (WS, $n = 5$), WAG/Rij (WR, $n = 5$), WAG/Rij-AGS (WRA, $n = 5$), and KM ($n = 5$) strains. Coronal brain sections (18 μm) were taken at four anatomical levels (+2.52, +1.92, –0.24, and –3.24 from the bregma) with a cryotome at –18°C. The sections were incubated with 1 mL of 50 mM Tris buffer (pH 7.5) containing 0.4 nM [3H]spiperone at 24°C for 1 h. Haloperidol (10^{-5} M) and ketanserin were used for binding. After the incubation, the sections were twice immersed in the Tris buffer at 4°C and distilled water for 2 s and dried with cold air. The sections were placed onto a tritium-sensitive film together with Amersham3H Microscale Autoradiography Standards® and exposed for 8 weeks. Digital images of the sections were processed using the ImageJ program. Structures were identified using an atlas [5]. All cortical areas were grouped into motor, sensorimotor, and cingulate regions without separation of the primary and secondary cortex in the analysis. A group with the factor Epilepsy included KM rats with audiogenic epilepsy, WAG/Rij rats with absence

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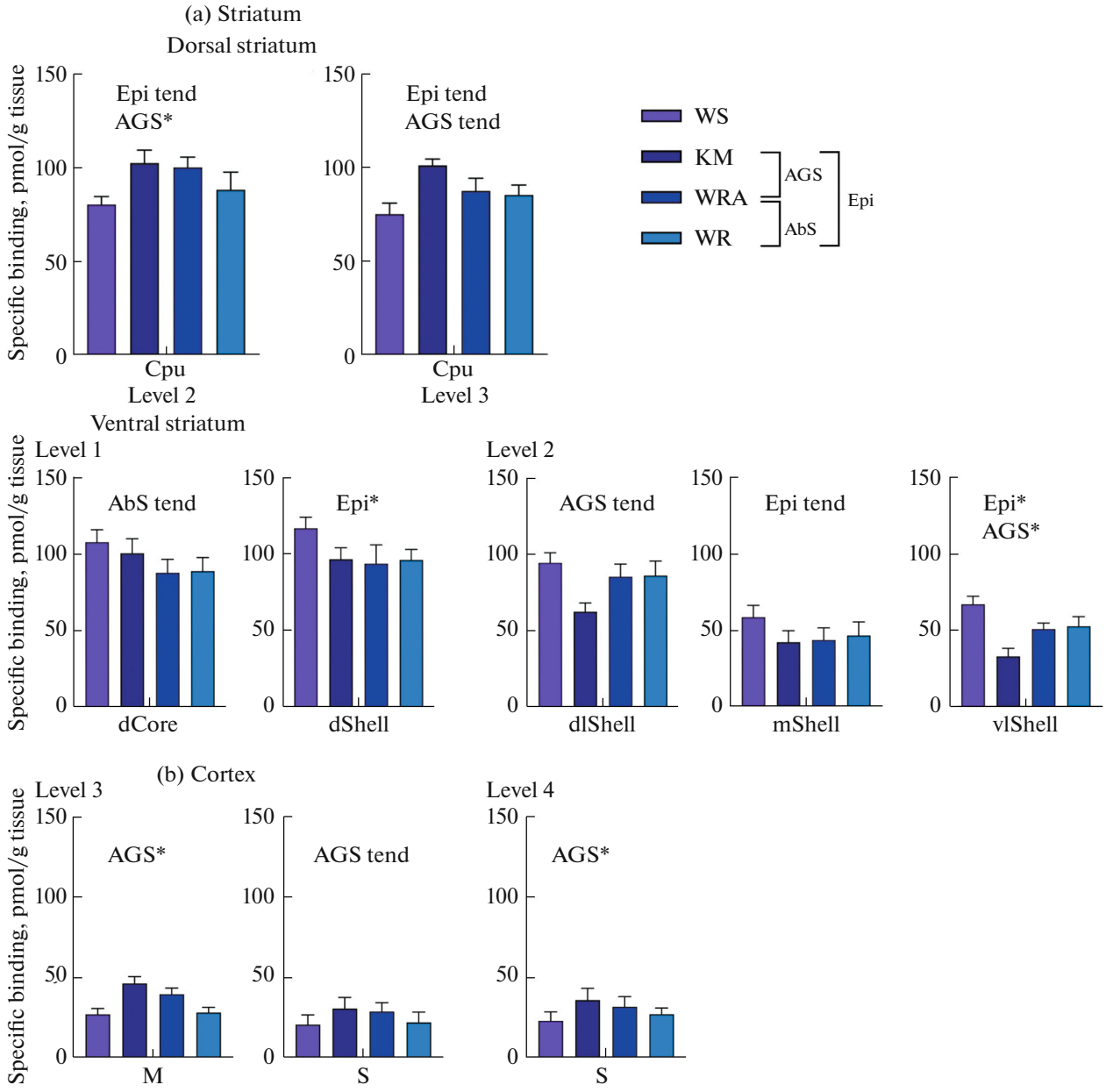

Fig. 1. Specific binding of [3H]spiperone to D2DR in the (a) striatum and (b) cortex, pmol/g tissue. Cpu, caudate putamen; Core, nucleus accumbens core; Shell, nucleus accumbens shell; d, dorsal; dl, dorsolateral; m, medial; vl, ventrolateral; M, motor cortex; S, somatosensory cortex. (*) $p < 0.05$, (**) $p \leq 0.01$. Factors: Epi, Epilepsy; AGS, Audiogenic Epilepsy; AbS, Absence Epilepsy.

epilepsy, WAG/Rij-AGS rats with combined (absence and audiogenic) epilepsy.

Statistical analyses were carried out using Statistica 14.0.0.15 (TIBCO Software). The D2DR binding density was analyzed by ANOVA GLM as described previously for the same rat cohort [6]. Two measurements were performed, and the results averaged. Contralateral measurements were used for missing values. Post-hoc analyses were performed using Fisher's test. The results were shown as mean ± standard error of the mean.

RESULTS AND DISCUSSION

Specific Binding to D2DR

1.1. General D2DR distribution. The highest D2DR binding density was observed in the dorsal striatum and the core and shell of the nucleus accumbens. D2DRs were additionally observed in the cortex, claustrum, and endopiriform nucleus, in agreement with published data [4].

1.2. Factor Epilepsy. Specific binding of the selective ligand with D2DR in the rat strains with genetic epilepsy (KM, WR, and WRA; $n = 15$) was significantly lower than in Wistar rats ($n = 5$) in the dorsal shell (dShell) of the nucleus accumbens at the first anatomical level (–21.3%; $p = 0.045$) and the ventrolateral shell (vlShell) at the second anatomical level (–35.9%; $p = 0.01$). The binding tended to be lower in the dorsal striatum (Cpu) at the second (+19%; $p = 0.083$) and third (+21.9%; $p = 0.93$) anatomical levels and the medial shell (mShell) of the nucleus accumbens (–29%; $p = 0.058$) (Figs. 1a, 2).

1.3. Factor Audiogenic Epilepsy. Specific binding of the selective ligand [3H]spiperone to D2DR on brain sections was compared between rats with (KM and WRA, $n = 10$) or without (WR and WS, $n = 10$) signs of audiogenic epilepsy. Rats with audiogenic epilepsy showed a higher binding in Cpu at the second anatomical level (+19.2%, $p = 0.039$), the motor cortex at the third level (+50.8%, $p = 0.0087$), and SmI at the fourth level (+39.5%, $p = 0.01$) and a lower binding in vlShell (–28.5%, $p = 0.01$). Tendencies to changes were observed in Cpu (+17.5%, $p = 0.086$) and SmI (+39.5%, $p = 0.06$) at the third anatomical level and the dorsolateral nucleus accumbens (–25.4%, $p = 0.067$) at the second level (Figs. 1b, 2).

1.4. Factor Absence Epilepsy. Rats with absence epilepsy (WR and WRA, $n = 10$) tended to have a lower specific binding to D2DR in the dorsal core of the nucleus accumbens (–21.4%, $p = 0.066$) as compared with rats without absence epilepsy (KM and WS, $n = 10$) (Figs. 1a, 2).

General D2DR distribution. The number and duration of spike-and-wave discharges have been shown to increase when rats with an imbalance of the mesocortical and mesolimbic dopaminergic systems are treated with pharmacological agents in experimental studies. The ligand–D2DR binding density has previously been compared between WAG/Rij and ACI rats. D2DR binding density was for the first time compared between normal (Wistar) rats and rats with basically different epileptic disorders. The highest binding density was observed in the dorsal and ventral striatum, the hippocampus, and the cortex in all rat strains; the finding agrees with earlier data [4]. We analyzed the three factors that might contribute to the specifics of the D2DR distribution in rats with genetically determined generalized epilepsy: the nonspecific factor Epilepsy; the factor Absence Epilepsy, which is specific for nonconvulsive epilepsy; and the factor Audiogenic Epilepsy, which is specific for convulsive epilepsy.

The effect of the factor Epilepsy. A lower D2DR density in the dorsal and ventrolateral regions of the nucleus accumbens shell was observed in rats with genetically determined epilepsy as compared with nonepileptic rats. The nucleus accumbens [7, 8] and, in particular, projections of dopaminergic neurons in the mesolimbic system [7] have been shown to play a modulating role in epilepsy. Neurons of both core and shell of the nucleus accumbens exert a modulating

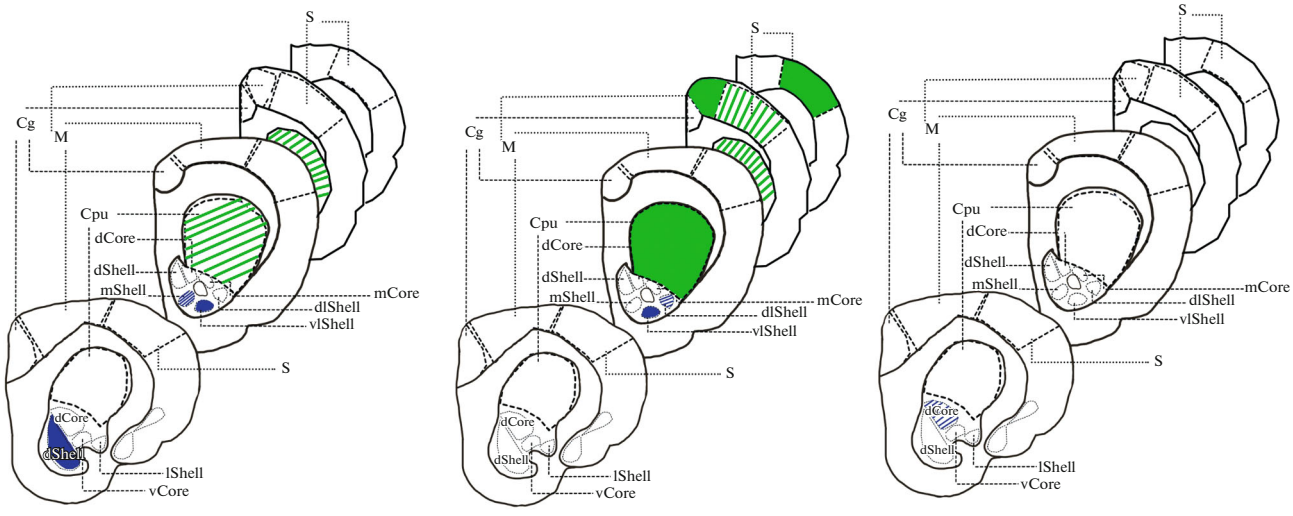

Fig. 2. Schemes of the brain structures under study. Decreases and increases are color coded blue and green, respectively; solid color indicates significant differences ($p < 0.05$) and crosshatching, a tendency ($p < 0.1$). The factors were Epilepsy (Epi), Audiogenic Epilepsy (AGS), and Absence Epilepsy (AbS); their presence or absence is indicated as 1 or 0, respectively. Cpu, caudate putamen; Core, nucleus accumbens core; Shell, nucleus accumbens shell; d, dorsal; l, lateral; dl, dorsolateral; m, medial; v, ventral; vl, ventrolateral; Cg, cingulate cortex; M, motor cortex; S, somatosensory cortex.

effect in epilepsy, the shell being more tightly associated with the limbic system [9]. Changes in the nucleus accumbens in epilepsy might be associated with neuronal degeneration, which occurs mostly in the nucleus accumbens shell [10].

The effect of the factor Audiogenic Epilepsy. The D2DR density in the caudate nucleus and motor cortical area in rats with audiogenic epilepsy was found to be significantly higher than in rats without the signs. A D2DR agonist has been observed to exert an anticonvulsive effect in the caudate nucleus in a pilocarpine model of temporal epilepsy [11]. The findings suggest a compensatory role in audiogenic seizures for the dopaminergic system. Pronounced motor excitation, which accompanies the first phase of audiogenic seizures (running with loss of visual control), probably leads to a subsequent increase in D2DR binding density in the motor cortex, the change being a step in activation of anticonvulsive systems in the brain. The brainstem and colliculi are known to play a major role in the pathogenesis of audiogenic epilepsy [12]. Unfortunately, necessary anatomical levels involving the regions of interest of these structures were not found in the sections included in this study. A marginal region of the surface gray layer of the superior colliculus (SuG) was detected at the level of –5.52 from the bregma. However, a corresponding trend (-28.3%, $p = 0.073$) was already observed in this small part of the structure.

The effect of the factor Absence Epilepsy. A lower D2DR density observed in rats with absence epilepsy as compared to rats without its signs supports the idea that dopaminergic dysfunction of the mesolimbic pathway acts together with dysfunction of the thalamocortical network. The core of the nucleus accumbens has been assumed to modulate the corticothalamic network because pharmacological agents injected in the region affect the number and duration of spike-and-wave discharges [7]. Higher expression of the D3 receptor mRNA has additionally been observed in the core of the nucleus accumbens in rats with genetically determined absence seizures [13]. A lower D2DR density in the region possibly point to insufficiency of the mesolimbic dopaminergic system of the brain in rats with absence seizures. Changes of the kind may underlie the pathogenesis of motivation and behavioral disorders characteristic of certain forms of epilepsy.

CONCLUSIONS

The D2DR density distribution was observed in the dorsal and ventral striatum and the cingular, motor, and sensorimotor regions of the cortex. The D2DR density in the core and shell of the nucleus accumbens in rats with the factors Epilepsy and Absence Epilepsy relative to the respective comparison groups. A higher D2DR density in the motor and somatosensory cortical regions was detected using the factor Audiogenic Epilepsy. The finding that the D2DR density decreases nonspecifically in the ventrolateral and dorsal shell of the nucleus accumbens suggests a common component for the neuronal chains involved in the pathogeneses of convulsive and nonconvulsive forms of epilepsy.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflicts of interest.

Statement on the welfare of animals. All experiments were performed in compliance with international guidelines for animal research. The study was approved by an Ethics Committee (Minutes no. 1-25022021 dated February 25, 2021).

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