

Update on the Role of Substantia Nigra Pars Reticulata in the Regulation of Seizures

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The substantia nigra pars reticulata (SNR) represents an endogenous seizure suppressing system, which may be targeted to develop treatments for generalized or multifocal epilepsies. This review summarizes the region-, age-, and sex-specific features of the SNR-based seizure-controlling network.

The substantia nigra pars reticulata (SNR), a midbrain structure that is a well-recognized component of motor control systems, also plays a critical role in the modulation of seizures (1,2). The SNR is the larger part of the substantia nigra proper, and contains mainly GABAergic neurons with high spontaneous firing rates. These neurons receive inputs from striatum by two distinct pathways (3). The striato-nigral direct pathway exerts GABA-mediated inhibitory effects on SNR neurons. The indirect striato-pallido-subthalamic-nigral pathway provides excitatory glutamatergic inputs from the subthalamic nucleus. During normal behavioral conditions, the indirect glutamatergic input seems to have an insignificant role in the regulation of SNR neurons (4), which may not be the case during seizures. Furthermore, a series of experimental studies strongly suggest that the role of the SNR in the control of seizures is different in male and female rats and also changes as a function of age.

The SNR plays an important transmitting role by directing information either back to the striatum or to the output structures of the basal ganglia (i.e., the thalamus, superior colliculus, and brainstem, via the pedunculo-pontine tegmental nucleus). The striato-nigro-striatal loop serves as an inhibitory feedback control for the activity of the SNR neurons. GABA release at the striato-nigral terminals or exogenous application of GABA

decreases the firing rate of the SNR GABAergic neurons (5,6). Either event results in a decrease of GABA release in the output structures, leading to their disinhibition (see Figure 1).

Role of the SNR in Seizure Control

The involvement of the SNR in seizures was recognized from metabolic mapping studies using ¹⁴C-2-deoxyglucose (2DG) in adult rats. Among brain structures, the increase in glucose uptake in the SNR during different types of seizures is especially striking. While the patterns of 2DG uptake vary in different seizure models, the SNR is always activated, especially during generalized seizures (7–10). A recent study showed that distinct regions of the SNR are activated sequentially during the evolution of a generalized seizure. The posterior part of the SNR (SNR_{posterior}) becomes involved just before the expression of the seizure, while the anterior part (SNR_{anterior}) is activated during the seizure (10).

Pharmacological studies using focal drug applications have contributed greatly to the understanding of how the SNR regulates the seizures. Most of these studies examined the role of the SNR_{anterior}. Treatments that decrease the activity of the SNR_{anterior} GABAergic neurons lead to attenuation of seizures. The original studies showed that precisely localized bilateral microinfusions of GABA_A receptor agonists, such as muscimol, bilaterally into the SNR produce anticonvulsant effects (1). In contrast, localized SNR microinfusions of the GABA_A receptor antagonist bicuculline have proconvulsant effects (11). In addition, suppression of the SNR glutamatergic input from the subthalamic nucleus by local microinfusions of glutamate receptor antagonists (e.g., AP7, an *N*-methyl-D-aspartate [NMDA] receptor antagonist) into the SNR decreases the firing rate of SNR neurons and, thus, induces anticonvulsant effects (12,13). The fact that seizure activity is sensitive to manipulations of both GABA and glutamate receptor systems within the SNR suggests that, unlike during normal behavioral conditions (4), the nigral glutamatergic inputs are actively involved during seizures. These results, together with data from lesion studies (14), demonstrate that the anticonvulsant effects are associated with decrements in the activity of the SNR_{anterior} GABAergic neurons leading to disinhibition of the output structures (5): the superior colliculus and the pedunculo-pontine tegmental nucleus (3). The disinhibition is essential for the anticonvulsant effect. Accordingly, bicuculline infusions in the superior colliculus or pedunculo-pontine nucleus are anticonvulsant but localized muscimol infusions or lesions are proconvulsant (15–18).

drive on the subthalamic nucleus, an important ascending projection of the pedunculo-pontine tegmental nucleus (38). This sequence of events is depicted as increases 2DG uptake in the subthalamic nucleus, another structure implicated in seizure control (10).

Region-Specific Regulation of Seizures by the SNR in Adult Female Rats

Less information is available on the role of the SNR during seizures in female rats. Interestingly, a recent kindling study showed that in females, muscimol infusions in the SNR anterior have less powerful anticonvulsant effects (39) than had been reported in male rats (40). This finding may explain the observation that SNR lesions in females have no effect on kindling-induced seizures (41). In vivo single-unit recordings show that neuronal activity is increased in the SNR posterior but not in the SNR anterior following amygdala kindling (42), suggesting that two functionally distinct regions also exist in females. The role of the SNR may be more prominent in generalized seizures. Muscimol infusions in the SNR anterior have anticonvulsant effects but no effect in the SNR posterior in flurothyl-induced generalized seizures (21). Indeed, the SNR anterior has higher expression of the $\alpha 1$ and $\gamma 2$ subunits of GABA_A receptor and of KCC2 mRNA as well as higher GABA levels per neuron, compared with the SNR posterior (25,27). Each SNR region also utilizes distinct networks following unilateral localized muscimol infusions (29). However, the most striking finding is that in contrast to male rats, in females, there is no proconvulsant muscimol-sensitive region (21).

Age-Specific Effects of the SNR in Seizure Control

Developmental studies show that the effectiveness of the SNR-based seizure-suppressing system is different in immature rats compared with adult rats. In contrast to adult male rats, in 2-week-old male pups, activation of the GABA_A receptors by muscimol has proconvulsant effects (43), and there is only one functional region at this age (19,21). In addition, the SNR GABA_B receptor system seems to play a more important role in developing male rats than in adult male rats. While in adult male rats infusions of the GABA_B receptor agonist baclofen or the receptor antagonist CGP 35348 have no effect on seizure threshold, in 2-week-old rats baclofen infusions have anticonvulsant effects, and CGP 35348 infusions are proconvulsant (44,45). Other developmental differences between developing male and adult male rats include lower expression of $\alpha 1$ and $\gamma 2$ subunits of GABA_A receptors (23), lower GABA content per neuron (25), and lower expression of KCC2 (27) in the male rat pups. Developmental studies in female rats also reveal that functionality of the SNR-based seizure-suppressing system changes

with age. As in 2-week-old male rats, muscimol infusions in the SNR of 2-week-old female rats do not distinguish between SNR anterior and SNR posterior. But in contrast to males, these infusions in females do not alter the seizure threshold (21).

Sex-Specific Effects of the SNR in Seizure Control Early in Life

In both male and female rats, the switch to the mature SNR with two distinct regions occurs around puberty (21). The timing corresponds to dramatic hormonal changes and may not be coincidental, indicating that the maturation of the SNR may be under the influence of sex hormones (21). Recent studies suggest that the SNR is equipped with both estrogen and androgen receptors, which are expressed at birth (46). During development, circulating sex hormones have organizing effects, leading to permanent differences between males and females (47). This fact explains the sex-specific SNR effects in seizure control, such as the previously cited difference in the effects of muscimol infusions into the SNR of 2-week-old male rats (proconvulsant) and female rats (no effect) (21). The crucial factor in the formation of the sex differences in the SNR is the presence of testosterone or its metabolites (estrogen and dihydrotestosterone), especially during early postnatal development. In male rats, depletion of testosterone by orchietomy immediately after birth leads to the female SNR phenotype while, in females, postnatal administration of testosterone leads to the male SNR phenotype (21). The male–female differences in seizure control seem to be associated with sex-specific differences in the GABAergic system within the SNR as well as in connectivity patterns (29). In the SNR of a male rat, the GABA content per neuron and GABA_A receptor $\alpha 1$ subunit mRNA expression is lower compared to females at the same age (25). The male SNR neurons express lower levels of the neuronal-specific KCC2 mRNA than females, which may explain the depolarizing responses to bath application of muscimol or synaptically released GABA, while female neurons respond by membrane hyperpolarization of the SNR GABAergic neurons (27,48). In addition, in 2-week-old rats, acute sex hormone administration regulates the expression of KCC2 in the SNR, further suggesting that the hormonal surges during development also may be responsible for the sex-differences in the modulation of seizures by the SNR-based system (49).

Conclusion

Recent advances in technology offer possibilities for new treatments of epileptic disorders. One promising procedure may be deep brain high-frequency stimulation (50). However, for a successful use of such a treatment in epilepsy, identification of structures controlling the general seizure activity and multifocal

epilepsies is essential. The SNR seems to be one of the promising regions as a target for treatments involving high-frequency stimulation (51) or focal drug delivery, which potentially could replace systemic antiepileptic therapy. Understanding the region-, sex-, and age-specific features of the SNR seizure-controlling network is important for developing precisely targeted therapies that would take into account the maturational state and gender-related factors.

Acknowledgments

Supported in part by grant NS-20253 from NINDS. SLM is a Martin A. and Emily L. Fisher Fellow in Neurology and Neuroscience.

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