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Sex-specific control of flurothyl-induced tonic–clonic seizures by the substantia nigra pars reticulata during development

Libor Velíšek [a,b,d,*], Jana Velíšková [a,b,d], Filippo S. Giorgi [a,1], Solomon L. Moshé [a,b,c,d]

[a] Department of Neurology, Albert Einstein College of Medicine, Bronx, NY 10461, USA
[b] Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461, USA
[c] Department of Pediatrics, Albert Einstein College of Medicine, Bronx, NY 10461, USA
[d] Einstein/Montefiore Epilepsy Management Center, Albert Einstein College of Medicine, Bronx, NY 10461, USA

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Abstract

The substantia nigra pars reticulata (SNR) plays an important age- and sex-specific role in control of clonic seizures. Its involvement in control of tonic–clonic seizures is contradictory. We investigated the role of the SNR in the tonic–clonic seizures induced in male, female and neonatally castrated male rats using flurothyl. In adult female rats, vaginal impedance determined the changes in progesterone/estrogen ratio. Rats at various postnatal ages received infusions of muscimol or vehicle in the SNRanterior or SNRposterior. Furthermore, in 15-day-old (P15) and adult male rats, ZAPA (a GABA(A) receptor agonist) or AP7 (an NMDA receptor antagonist) was infused. The developmental profile of tonic–clonic seizure threshold differed between male and female rats possibly due to early postnatal testosterone surge in male rats. On the other hand, changing estrogen/progesterone ratio in cycling adult female rats had no effect on seizure threshold. Intranigral muscimol had proconvulsant effects on tonic–clonic seizures only in immature rats, and this effect was dependent on the perinatal testosterone surge. ZAPA had anticonvulsant effects in P15 rats but was not effective in adult rats. Only AP7 had anticonvulsant effects in both adult and P15 rats. Results indicate that thresholds for flurothyl-induced tonic–clonic seizures develop under the control of postnatal testosterone. Although GABAergic inhibition in the SNR affects tonic–clonic seizures in developing rats, only the NMDA antagonist had consistent anticonvulsant effects throughout development.

Keywords: GABA; Muscimol; ZAPA; NMDA; AP7; Flurothyl; Seizure; Testosterone; Estrous cycle

Introduction

In both humans and experimental animals, seizures are sensitive to hormonal milieu (Herzog et al., 1997; Velíšková, 2006b). Several epileptic syndromes show gender-specific features (Wallace, 1991), and there is higher incidence of seizures in males compared to females (Hauser et al., 1993). This may be related to the presence of sex differences within brain regions involved in seizure initiation or suppression. One of these structures may be the substantia nigra pars reticulata (SNR).

Previous studies have indicated that pharmacologic manipulations within the SNR affect predominantly clonic (i.e., forebrain) and absence seizures, but not tonic–clonic (i.e., brainstem) seizures (Deransart et al., 2001). However, initial reports on the SNR seizure control found anticonvulsant effects of the microinfused opiates, muscimol and substance P antagonists in the SNR against maximal electroshock seizures (Garant and Gale, 1985, 1986, 1987). Seizures induced by maximal electroshock are tonic–clonic and probably of brain stem origin. These studies thus indicate that the SNR may participate in the control of tonic–clonic seizures. This notion is supported by our metabolic mapping using [^{14}C]2-deoxyglucose (Velíšková et al., 2005) indicating involvement of the SNR during tonic–clonic seizures.

* Corresponding author. AECOM, K314, 1410 Pelham Parkway South, Bronx, NY 10461, USA. Fax: +1 718 430 8899.
E-mail address: velisek@aecom.yu.edu (L. Velíšek).
[1] Current affiliation: Department of Neurosciences, section of Neurology, University of Pisa, Via Roma 67 I56126, Pisa, Italy.

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The role of SNR in control of clonic seizures (Iadarola and Gale, 1982) is region- (Moshé et al., 1994) and sex-specific (Velíšková and Moshé, 2001). In the adult rats, the SNR consists of two distinct subregions (SNRanterior and SNRposterior) (Moshé et al., 1994). Infusions of GABA(A)ergic drugs in these subregions differentially affect expression of clonic seizures (Fan et al., 1997, 2000; Moshé et al., 1994; Thompson et al., 2000). In the adult male rat, symmetrical bilateral microinfusions of muscimol (a GABA(A) receptor agonist) in the SNRanterior have anticonvulsant effects against clonic flurothyl-induced seizures. However, in the SNRposterior, similar muscimol infusions mediate proconvulsant effects (Velíšková and Moshé, 2001). The SNR subregions develop in age- and sex-specific fashion. In 15- to 21-day-old (P15–21) male rats, there is only a single region in the SNR, and here muscimol infusions mediate only proconvulsant effects (Velíšková and Moshé, 2001). The SNR region, which from P30 on mediates anticonvulsant effects of muscimol (SNRanterior), can be distinguished by P25; at this age, muscimol infusions in the SNRanterior have no effect on seizures. In female rats, muscimol in the SNR between P15 and 21 has no effects on flurothyl-induced clonic seizures (Velíšková and Moshé, 2001), and the anticonvulsant subregion is already active on P25. All these studies use as an endpoint clonic flurothyl-induced seizures, which emerge from the forebrain (Browning, 1985; Browning and Nelson, 1986).

Chemically induced acute primary generalized motor seizures in experimental animals usually consist of clonic and tonic–clonic seizures (Velíšek, 2006). In rats, flurothyl induces both types of seizures (Velíšek, 2006): 1. clonic seizures consist of clonic convulsions of forelimbs and facial muscles with preserved righting ability. If the tonus of axial muscles alters the erect posture, the rat actively and quickly regains upright position. The entire clonic seizure duration counts in seconds to tens of seconds (Velíšková, 2006a). 2. In adult rats, tonic–clonic seizures occur with a significant time lag after clonic seizures. In immature, 15-day-old (P15) rats, tonic–clonic seizures may follow immediately after a clonic seizure episode. Tonic–clonic seizures begin with wild running followed by a loss of posture (righting reflex) followed by a short tonus and a long clonus of all four limbs occurs (Velíšková, 2006a). Lesion studies have demonstrated that precollicular brain stem transection abolishes the occurrence of clonic, but not tonic–clonic seizures (Browning, 1985; Browning and Nelson, 1986). Thus, the brain stem regulates the occurrence of tonic–clonic seizures (Browning, 1985). Since tonic–clonic seizures may use differential control circuits, the compartmentalization of the SNR may not apply in a similar fashion as in the clonic seizure control.

Therefore, we examined the SNR control of tonic–clonic seizures induced by flurothyl by focal SNR microinfusions of GABA receptor agonists. To determine the development of the SNR subregions, the microinfusions of muscimol were initially aimed to either anterior or posterior part of the SNR in all age groups. For a comparison, an NMDA receptor antagonist was also included because NMDA receptor antagonists effectively suppress tonic–clonic seizures after systemic administration (Velíšek et al., 1990).

Materials and methods

Animals

Experiments were reviewed and approved by the IACUC and carried out according to the Revised *Guide for the Care and Use of Laboratory Animals* (NIH GUIDE, Volume 25, Number 28, August 16, 1996). Several groups of developing Sprague–Dawley rats of both sexes (Taconic Farms, Germantown, NY, USA) were used at ages 15, 21, 25, 30 and 35 postnatal days and P60 (adult). Developmentally, these rats approximately correspond to human infants (P15), children (P21 and P25) and to peripubertal age (P30 and P35) and adults (PN60) (Avishai-Eliner et al., 2002; Gottlieb et al., 1977). Since puberty occurs approximately between P32 and P45 in females and between P36 and P55 in males with a variable onset in individual rats (Ojeda and Urbanski, 1994), we avoided testing during this interval if possible. Our previous data indicated that the SNR control of clonic seizures was sex-specific (Velíšková and Moshé, 2001). Therefore, female rats were also included in this study as separate groups at P15, P21, P25, P30 and P60. Due to cycling hormonal changes, vaginal impedance was measured in all adult female rats immediately before the experiment. For further investigation of sex specificity, the groups of neonatally castrated male rats were used. All immature rats were housed with lactating dams until weaning (on P21). All rats were kept in our AAALAC-approved animal facility on a 12 h light:12 h dark cycle (lights on at 7:00) with free access to food and water.

Intracranial surgery

Rats were subjected to the surgery 2 days before testing (i.e., on P13, P19, P23, P28, P33 and P58) using our established protocol and coordinates (Velíšková and Moshé, 2001) listed in the Table 1. Guide cannulae (Plastics One) were stereotaxically implanted into the SNR bilaterally under deep anesthesia with a mixture of ketamine (70 mg/kg ip) and xylazine (7 mg/kg ip). Because the SNR has two functional regions (SNRanterior and

Table 1
Stereotaxic coordinates for cannula placement as a function of age (from bregma in millimeters)

AGE	SNRanterior			SNRposterior		
	Anteroposterior	Lateral	Depth	Anteroposterior	Lateral	Depth
PN58	5.3	4.0	7.0	5.8	4.0	7.1
PN33	5.3	3.8	6.8	5.6	3.8	6.9
PN28						
PN23	5.2	3.5	6.5	5.5	3.5	6.7
PN19	5.2	3.5	6.4	5.5	3.5	6.5
PN13	5.2	3.5	6.0	5.5	3.5	6.1

Ages refer to the age 2 days prior to testing, when the surgery was performed. Microinfusions were initially aimed into the anterior or posterior part of the SNR because we did not know the development of the SNR control subregions for tonic–clonic seizures. Coordinate system uses 15° cannula angle from the sagittal plane, the depth was measured from the skull surface. Incisor bar was set at −3.5 mm. It should be emphasized that these coordinates refer to the guide cannula implant. The internal cannula (the infusion device) protruded 1 mm beyond the tip of the guide cannula (add 1 mm to the depth).