Absence seizures during pregnancy in WAG/Rij rats

Elena A. Tolmacheva[a], Serguei A. Chepurnov[a], Nina E. Chepurnova[a],
Yakov A. Kochetkov[b], Gilles van Luijtelaar[c],*

[a]*M.V. Lomonosov Moscow State University, Biological Faculty, Moscow, Russia*
[b]*Federal Center of Psychoendocrinology, Moscow Institute of Psychiatry, Moscow, Russia*
[c]*Nijmegen Institute for Cognition and Information, Biological Psychology, University of Nijmegen, PO Box 9104, 6500 HE, Nijmegen, The Netherlands*

Abstract

Spontaneously occurring spike-wave discharges (SWDs) and serum concentrations of ovarian steroid hormones were investigated before, during and after pregnancy in WAG/Rij rats, a rat strain with genetically determined absence seizures. Eight groups of rats were included in the assays of progesterone and estradiol: rats at diestrus, at various days of pregnancy and at lactating days. The number of SWDs in cortical EEG of WAG/Rij rats was decreased from the 3rd up to the 18th day of pregnancy and subsequently increased to control level. Thereafter, a new decrease was found 2–3 days after parturition. Serum concentration of progesterone was threefold increased at the 3rd day of pregnancy, remained elevated until the 18th day of pregnancy and returned to control values before delivery. Over measured days, estradiol was significantly elevated only at the 18th day of pregnancy.

Results demonstrate that physiological conditions induced by the state of pregnancy lead to suppression of occurrence of SWDs. Changes in plasma progesterone concentration correspond to the changes in number of SWDs: an increased level of progesterone during pregnancy is accompanied by a decreased number of SWDs, while a decrease in circulating progesterone before parturition is paralleled by an increase of SWDs. Of interest, the relationship between SWDs and concentration of progesterone found during pregnancy is diametrically opposite to results obtained in acute administration studies of progesterone in nonpregnant animals.

1. Introduction

Bilateral, synchronized spike-wave discharges (SWDs) represent a hallmark of a specific form of idiopathic non-convulsive types of epilepsy, such as absence epilepsy. Spontaneously occurring spike-wave activity mediated in a cortico–thalamo–cortical network is abundantly present in the cortical electroencephalogram of several strains of rats, such as WAG/Rij and GAERS. The WAG/Rij strain of rats is considered a well-validated genetic model [1,2] which offers among others, a unique possibility to research occurrence of absence seizures in a variety of physiological conditions. Fluctuations in plasma and brain concentrations of steroid hormones and their neuroactive metabolites over different stages of reproductive cycle might play an important role in the modulation of neuronal excitability [3–5]

and therefore result in alterations in emotional state, sleep pattern and seizure threshold.

Several studies indicate a possible physiological role of ovarian steroid hormones in absence epilepsy [6–8]. In a single-case human study, absences were aggravated after administration of progesterone [6]. In an animal model, systemic acute administration of progesterone, but not estradiol, increased the number and total duration of SWDs in WAG/Rij rats and injection of an antagonist of the intracellular progesterone receptors RU 38486 had no effect on SWDs and did not block the stimulatory effect of progesterone [7]. The latter data suggest that the effects of progesterone were likely due to membrane transmitter receptors. Investigation of spontaneously occurring SWDs during the estrous cycle, again in WAG/Rij rats, has revealed an increase in the number of SWDs at proestrus day at the time of day in which the level of progesterone is enhanced [8]. These data suggest not only a clear role of progesterone in generalised absence epilepsy but also

* Corresponding author. Tel.: +31-24-3615621; fax: +31-24-3616066.
E-mail address: g.vanluijtelaar@nici.kun.nl (G. van Luijtelaar).

demonstrate that, in contrast to what has been found in convulsive types of epilepsy [9,10], progesterone may exacerbate epileptic activity of the SWD type. The precise mechanisms of this involvement are still unclear. Earlier, it was suggested that the increase of SWDs after acute administration of progesterone in WAG/Rij rats was due to the GABAmimetic action of its active metabolite allopregnanolone [7]. This was recently confirmed in an experiment in which the increase induced by progesterone was antagonized by finasteride, a 5alpha-reductase inhibitor, which itself did not have an effect on SWDs [11]. These data suggest that some of the central effects of progesterone on SWDs are exerted through the GABAergic system. A major metabolite of progesterone, allopregnanolone, is an allosteric modulator of GABA$_A$ receptor which may facilitate GABA-mediated response by an increase in both Cl$^-$ channel opening time and frequency [12]. Several studies have indicated that long-term treatment with neuroactive steroids, including genomic component, may lead also to various changes in density, plasticity and sensitivity to modulation induced by benzodiazepines and neurosteroids itself [4,5,13–15]. All these changes are region specific and can be different for the low- and high-affinity binding sites [1,13–15].

Variety of changes which could be induced by genomic effects of progesterone makes it difficult to predict their ultimate impact on characteristics of thalamo–cortical circuits to generate SWDs. Pregnancy is a dominant physiological state, which is associated with striking changes in hormonal milieu and offers a unique model to study the chronic effects of large (but physiological) changes in progesterone in an experimental way [16].

Present study was designed to investigate the possible functional relation between changes in plasma concentrations of progesterone and estradiol and level of occurrence of SWDs during pregnancy and after delivery.

2. Materials and methods

Adult female WAG/Rij rats with body weight of 190–210 g were used as experimental subjects. They were housed five per cage under artificial 12-h light, 12-h dark cycle (lights on from 0800 to 2000 h) at a constant room temperature. All manipulations with animals were according to the locally approved codes of practice for the care and use of animals for scientific purposes.

Stage of estrous cycle (diestrus, proestrus or estrus) was determined from daily vaginal smears taken between 0900 and 1200 h for 2 weeks. Only rats exhibiting at least three regular cycles with a duration of 4–5 days were included in the study. For the induction of pregnancy, females were caged with males on the evening of proestrus. Mating was verified by observation of spermatozoa in the vaginal smear taken next morning, which was designated as Day 0 of pregnancy. Dams were housed individually from the

third week of pregnancy onwards. The duration of pregnancy in WAG/Rij rats was found to be 21 days.

Eight groups of rats were included in progesterone and estradiol assay: diestrus rats, rats at Days 3, 7, 15, 18 and 20 of pregnancy and lactating rats, 1 and 3 days after delivery. Samples of blood from the v. jugularis were taken between 1200 and 1400 h. Sodium citrate (0.11 Ì) in proportion 1:9 was used as an anticoagulant. Blood was centrifuged at $1000 \times g$ for 15 min, after which the plasma was frozen until assayed for steroid hormones. The concentration of estradiol and progesterone in all samples was simultaneously determined with ELISA kits to determine the concentrations (Diagnostics, USA).

For EEG study, 10 animals were provided, under Nembutal anesthesia (40 mg/kg ip), with a standard EEG electrode set with coordinates: AP +2.0, L 3.0 for active, AP − 6.0, L 4.0 for the indifferent electrode, and the earth electrode was placed in the cortex of the cerebellum. Following surgery, rats were allowed to recover for at least 1 week. All rats were first familiarized with the recording and then were adapted to experimental cage during the first hour before each recording. During this study, the cortical EEGs were recorded between 1700 and 2000 h at 2 days before (this was considered as baseline), during various days of pregnancy and at third postpartum day. The dams were not separated from their pups during the EEG session. The EEGs were amplified, filtered between 1 and 100 Hz, and stored for off-line analyses. The hourly number and total duration of SWDs per hour were counted according to standard criteria.

Although it is acknowledged that there is a 5- to 7-h delay between EEG and blood samplings for steroid assays and that both variables show a circadian pattern, it can be remarked that there is a high positive correlation between the hourly number of SWDs during all hours of the day [17]. Moreover, unpublished data showed that differences between strains in concentrations of hypothalamo–pituitary–adrenal (HPA)-axis hormones were stable across a 12-h period.

The analyses of variance (ANOVAs) statistic for independent (progesterone and estradiol plasma levels) and repeated (number of SWDs) measurements were used, if necessary, followed by Newman–Keuls post hoc comparisons between days, a significant level of $P < .05$ was chosen for all tests.

3. Results

The data for the level of SWDs along over the course of pregnancy and after delivery are presented in Fig. 1. Obtained results demonstrate considerable changes for the number of SWDs in WAG/Rij rats during pregnancy. The ANOVA for repeated measurements has revealed significant day effects for hourly number of SWDs [$F(8,40) = 55.75$,
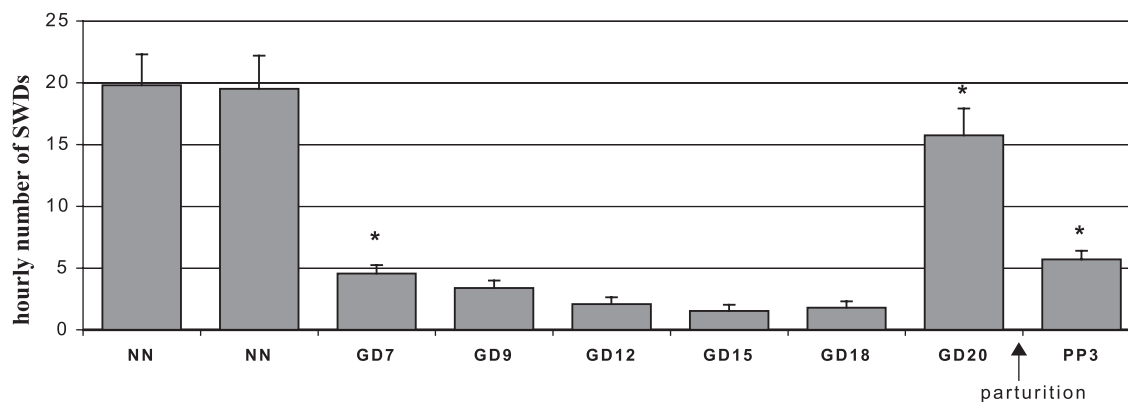
Fig. 1. The number (mean and S.E.M.) of SWDs per hour before, during and after pregnancy in WAG/Rij rats. NN—base line days, GD—gestation day, PP—postpartum. * Significant ($P<.05$) increase/decrease compared to previous day.

$P<.000$]. A significant constitutive suppression of SWDs generation was found from the 7th day onward until the 18th day of pregnancy. However, level of SWD activity was progressively increasing up to baseline values for 2–3 days before the delivery while it was found to be decreasing anew on 2–3 postpartum days.

Marked changes in circulating steroids concentration during pregnancy were found as presented in Table 1. Significant day effects for progesterone [$F(7,20)=164.29$, $P<.000$] were found. The serum concentration of progesterone was significantly increased from the 3rd day onwards and remained elevated until the 18th day of pregnancy compared with diestrus day. A progressive and significant increase in serum progesterone level was found on the 7th and the 18th day of pregnancy compared to previous measured days (3rd and 15th days, correspondingly). Thereafter and immediately before delivery, on the 20th day, plasma concentration of progesterone returned to control values, remained unchanged on 1 day after delivery, and then it was found to increase again on 3rd postpartum day. The time course of changes in the occurrence of SWDs during and after pregnancy and circulating progesterone concentration were in parallel over this period of time. This may suggest a functional relationship between SWDs and progesterone. In addition, a day effect for estradiol was found [$F(7,20)=4.02$, $P<.01$]. There were no considerable changes in plasma estradiol concentration during pregnancy with a single exception. A significant elevation in estradiol concentration compared to diestrus day and all other measured days of pregnancy and postpartum days was found only at Day 18 of pregnancy.

4. Discussion

This study was aimed to establish the level of SWD occurrence during pregnancy and after parturition and to investigate a possible functional relation between changes in spike-wave activity and changes in endogenous plasma steroid concentrations over these periods. Obtained results clearly demonstrate that the level of occurrence of SWDs is constitutively decreased in WAG/Rij rats during pregnancy. Under normal conditions, the daily number of SWDs in 6-month-old rats is more or less stable. This is the first report demonstrating a marked decrease in basal levels in occurrence of SWDs in a special physiological condition, such as pregnancy. As expected, major changes during pregnancy, including a rapid increase after onset of pregnancy, a maximum at Day 18 and a rapid decrease and a smaller postpartum increase, were found for serum progesterone level that were consistent with those observed in the literature [4,18,19]. Plasma estradiol level was significantly enhanced only at Day 18 of pregnancy; its increase at that specific time was also mentioned by Weizman et al. [18], while strain-related differences in the time course of estradiol plasma level during pregnancy were found in the last few days before parturition [19]. The increased level of progesterone, starting at Day 3 up to Day 18 of pregnancy, was accompanied by a decrease in the number of spontaneously occurring SWDs. The sudden decrease in the concentration of progesterone at 3 days before parturition was also accompanied by a change in SWDs, but now by an increase. Based on the data obtained in the present experiment, it can be assumed that during pregnancy in WAG/Rij rats, an increase in concentration of progesterone is paral-

Table 1
Plasma progesterone and estradiol concentrations (mean and S.E.M.) on diestrus day, 3rd, 7th, 15th, 18th and 20th day during pregnancy and on 1st and 3rd postpartum (PP) days

	Diestrus1 (n=6)	3rd day (n=3)	7th day (n=5)	15th day (n=3)	18th day (n=3)	20th day (n=3)	1st PP day (n=3)	3rd PP day (n=4)
Estradiol in nm/l	46 ± 7	50 ± 8	54 ± 10	59 ± 2	76 ± 9	41 ± 13	49 ± 15	31 ± 25
Progesterone in nm/l	42 ± 14	139 ± 3	166 ± 4	159 ± 7	180 ± 5.0	46 ± 15	36 ± 6	83 ± 2

leled by a decrease in number of SWDs and a decrease in concentration by an increase in SWDs. Therefore, it seems the relationship between progesterone and SWDs during pregnancy is opposite to what has been found in a few acute dose–effect studies of progesterone in male and female WAG/Rij rats and during the estrus cycle in female WAG/Rij rats [7,8,11].

In contrast to the short-term effect in the acute dose–response studies, the long-term steroid effects that are studied here involve delayed genomic effects mediated by citosolic receptors, which are widespread in the brain, mainly in the cortex and structures of the limbic system [20,21]. These citosolic receptors are involved in the regulation of GABAergic system functioning. In fact, constitutive changes in plasma and brain concentrations of progesterone and its active metabolites during pregnancy were found to be in association with changes in density, sensitivity and plasticity of GABA$_A$ receptor complex [4,18,22]. Another mechanism for regulation of GABA$_A$ receptor sensitivity to modulation is based on balance between endogenous phosphate and protein kinase C activity [23]. The shift of this balance induced by oxytocin before parturition led to insensitivity of GABA$_A$ receptors to allopregnanolone in supraoptic nucleus [24]. On the other hand, an up-regulation of GABA$_A$ receptors in cortical neurons and a down-regulation of these receptors in the ventral lateral thalamus were found after repeated administration of progesterone in the female hamster [15]. These areas form the key players in thalamo–cortical oscillations of the SWD type [25,26]. Considering the diminished GABAergic intracortical inhibitory mechanisms in WAG/Rij rats in normal condition [27], a positive modulation of GABA receptors in cortical neurons could prevent the hyperexcitability of the cortex, which is postulated as a major factor controlling the occurrence of SWD [28,29]. It is therefore assumed that increased functioning of GABA in the cortex and simultaneously down-regulation of GABA$_A$ receptors at the lateral basal complex of the thalamus might cause opposite effects during pregnancy as under normal circumstances when a positive relation between progesterone and SWD activity was found.

In addition, neuroactive steroid hormones are able to modulate not only the GABAergic system but also the glutamatergic (NMDA), cholinergic and opioid system [30], which were shown to be involved in absence epilepsy [31–33] and therefore these influences cannot be ruled out to play a role. Furthermore, pregnancy and lactation are physiological conditions associated with striking alterations in the hormone milieu and metabolism, also largely varying between different strains of rats [19]. It may be that various hormones have indeed direct or indirect effects on pathogenesis of absence epilepsy. To our knowledge, for only a few hormones, such as prolactin, estradiol and HPA-axis-related hormones, were the effects on absence seizures examined. Under physiological conditions and after systemic administration, no effects of estradiol or its antagonist on

SWDs were established in WAG/Rij rats [7,8]. The effects of prolactin on absence seizures were not previously described; however, what was known was that prolactin-releasing peptide suppressed absence seizures in GAERS [34]. However, because prolactin plasma level is substantially decreased during pregnancy [19], it is not likely that its effects contribute extensively to the decrease of occurrence of SWDs during pregnancy, although its contribution cannot fully be excluded. Fluctuations in plasma corticosterone levels and other HPA-axis hormones as found during pregnancy [19] might also be implicated in the regulation of the occurrence of SWDs because concentrations of ACTH and corticosterone were found to be correlated with the number of SWDs in normal circumstances [35]. Finally, effects of HPA-axis-related hormones on SWDs were established in a genetic mouse model of absence epilepsy [36].

It can be concluded that a constitutive decrease in the number of SWDs can be found during the state of pregnancy. Therefore, it seems that during pregnancy, the fetus of genetically epileptic rats is protected. During pregnancy, levels of progesterone and other hormones are chronically and seriously changed. A different relation between progesterone and SWDs was established here than in three earlier studies. It is thought that opposite effects on GABA receptors in the cortex and the thalamus, which may emerge only after repeated injections and not after acute ones, might explain the negative relation between levels of progesterone and SWDs. Whether this is sufficient reason for opposite effects on SWD presence between acute injection and chronic endogenous elevation in progesterone level during pregnancy is not immediately clear.

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