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Comparison of Cortical Epileptic Afterdischarges in Immature Genetic Absence Epilepsy WAG/Rij Rats with Those in Two Other Strains (ACI and Wistar)

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Summary: The aim of this study was to examine the development of cortical epileptic afterdischarges (ADs) in genetic absence epilepsy WAG/Rij rats, and to compare them with two strains with minimal incidence of spike-and-wave (SW) episodes (ACI and Wistar). Epileptic ADs were elicited by stimulation of sensorimotor cortex in 12-, 18-, and 25-day-old rats of the three strains. The threshold current intensities were established for movements accompanying stimulation, for ADs of the SW type and accompanying clonic seizures and for transition into limbic type of ADs (characterized by behavioral automatisms). Individual groups were formed by 7–12 rats. There were no differences among the three strains in the thresholds for elicitation of stimulation-bound movements. In contrast, WAG/Rij and ACI rats exhibited easier elicitation of SW ADs than Wistar rats at the age of 18 and 25 days. There was no difference among the three strains in transition into the limbic type of ADs in 18- and 25-day-old rats. Lower thresholds for SW ADs in 18- and 25-day-old WAG/Rij and ACI rats in comparison with Wistar rats are in agreement with our data from adult animals as well as with development of pharmacologically induced models of absence seizures. The failure to find a specific difference between WAG/Rij rats and the other two strains might indicate a difference in generation of SW episodes and SW cortical AD.
Key Words: Absence seizures—Epileptic afterdischarges—Cerebral cortex—Electrical stimulation—Ontogeny—Rat.

Human absences represent an age-bound type of seizures characterized by short loss of consciousness and spike-and-wave (SW) rhythm in the EEG; they appear in late preschool- and school-age children (Stefan and Snead, 1997). There are two genetic models of absence epilepsy in rats characterized by spontaneous appearance of episodes of SW rhythm—GAERS and WAG/Rij (for review Depaulis and van Luijtelaar, 2006). It was found that these episodes start in restricted part of the cerebral cortex of WAG/Rij rats (Meeren et al., 2002) supporting thus a primary role of neocortex in generation of the SW rhythm (van Luijtelaar and Sitnikova, 2006). In agreement with this finding is easier formation of GABA-withdrawal cortical focus in GAERS than in outbred Wistar rats (Brailowski et al., 1999) as well as a recent demonstration of higher excitability of slices of somatosensory cortex from WAG/Rij rats in comparison with cortex of Wistar animals (D'Arcangelo et al., 2006). SW episodes are found not only in the two strains mentioned above, but also in other inbred (including ACI rats commonly used as controls in studies of WAG/Rij rats—Inoue et al., 1990) and outbred (Wistar) strain (Chocholová, 1983), the differences are only quantitative. Using cortically induced epileptic afterdischarges (AD) we found an easier induction of SW (ADs), that is, an increased excitability of sensorimotor cortex not only in adult WAG/Rij but also in ACI strain in comparison with Wistar rats (Tolmacheva et al., 2004). On the other hand WAG/Rij rats needed lower stimulation current intensities than the other two strains to spread the epileptic activity into the limbic structures (Tolmacheva et al., 2004).

There is a contradiction between the ontogeny of SW episodes (the only developmental study performed in GAERS was able to register SW episodes since the age of 40 days (Vergnes et al., 1986) and pharmacological models of absence seizures demonstrating SW rhythm since the third postnatal week in Wistar rats (Schickerová et al., 1984; Snead, 1994). Therefore we decided to study development of cortical excitability of the three rat strains (WAG/Rij, ACI, and Wistar) by means of epileptic ADs elicited by stimulation of the sensorimotor cortex and compare the results with data from adult animals as well as with data on development of SW activity. We expected

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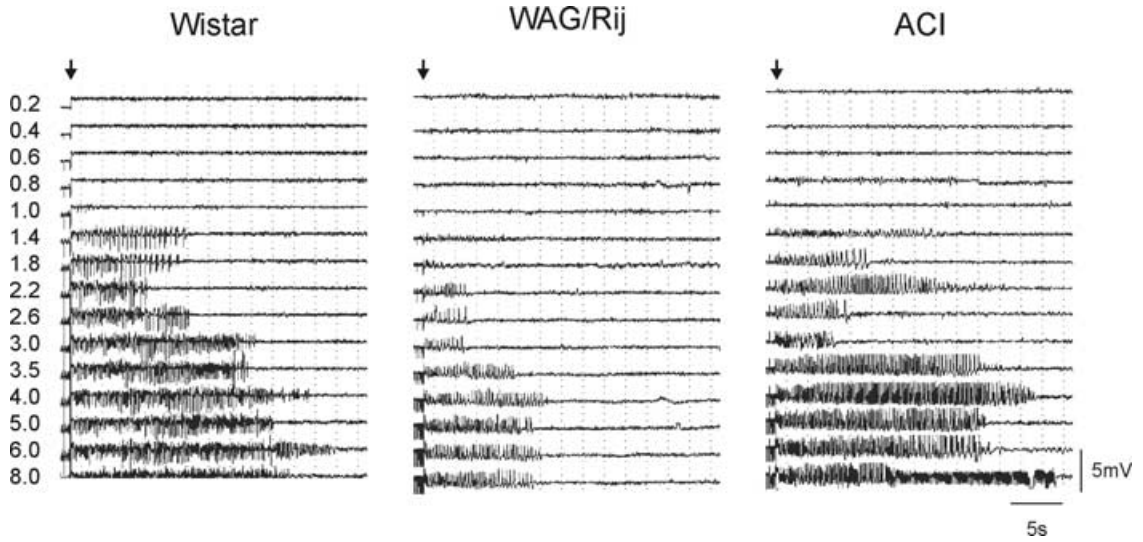

FIG. 1. Original recordings of afterdischarges in 18-day-old rats from Wistar, WAG/Rij and ACI strains (from left to right). Individual leads in each section demonstrate activity of the left frontal area. Recordings demonstrate the last second of stimulation (an arrow marks the end of stimulation) and 39 s after stimulation. Individual rows show increasing stimulation intensities—from top to bottom 0.2, 0.4, 0.6, 0.8, 1, 1.4, 1.8, 2.2, 2.6, 3, 3.5, 4, 5, 6, and 8 mA. Time mark 5 s, amplitude calibration 5 mV.

a marked increase of cortical excitability between the 12th and 18th postnatal day in agreement with the appearance of spike-and wave EEG rhythm elicited by convulsant drugs (Schickerová et al., 1984; Snead, 1994; Zouhar et al., 1989) or by thalamic stimulation (Mareš et al., 1982). This expected result could indicate different mechanisms generating SW rhythm in acute models and in genetic models of absence seizures.

METHODS

The experiments were approved by the Animal Care and Use Committee of the Institute of Physiology to be in agreement with European Community Council directives 86/609/EEC.

Three age groups (12, 18, and 25 days old) of the three rat strains (WAG/Rij, ACI and Wistar) were studied. Flat cortical silver electrodes ($<1\times1$ mm) were implanted epidurally under ether anesthesia. Stimulation electrodes were localized over right sensorimotor area (AP -1 and $+1$, L 2 mm), registration electrodes over left sensorimotor, parietal, and visual areas and over right visual area. Reference electrode was in nasal bone, ground in the occipital bone. Electrodes were fixed with a fast curing dental acrylic. Surgical preparation lasted 10–12 min, then ether anesthesia was interrupted and the animals were allowed to recover for at least 1 h. Their body temperature was maintained by means of a pad heated to 34°C, that is, to the temperature in the nest.

EEG activity was digitalized at a rate of 500 Hz and saved on the hard disc (Kaminskij Biomedical Research Systems, Prague). Behavior of animals was coded directly into the recording. Epileptic ADs were elicited by stimulation with 15-s series of 1-ms biphasic pulses at 8-Hz frequency. Interval between the subsequent stimulations was at least 10 min, current intensity was increased from 0.2 to 15 mA. Individual age groups were formed by 7–12 rats with equal number of male and female rat pups (with the exception of one group with $N = 7$). No significant differences between the two sexes were found in any strain and age groups, therefore the results were put together.

Threshold intensities of stimulation current were estimated for movements directly elicited by stimulation, for ADs of the SW type and their behavioral correlate (clonic seizures) and for transition into the second, limbic type of ADs accompanied by behavioral automatisms (mixed type, for details (Mareš et al., 2002; Mareš and Kubová, 2006). Duration of all ADs was measured and average values for individual stimulation intensities were calculated. ANOVA with subsequent pairwise comparison by Tukey's test (SigmaStat, SPSS Inc., Chicago, IL) was used for statistical evaluation. The level of statistical significance was set at 5%.

RESULTS

Movements bound to stimulation, SW ADs, and clonic seizures were elicited in all animals. Transition into the limbic type of ADs was observed in majority but not in all rats. There were no differences in EEG pattern of ADs as well as in frequency of sharp graphoelements among the three strains (Fig. 1); it was described in detail in Wistar rats (Mareš et al., 2002).

The three strains exhibited different developmental profiles. Developmental differences in Wistar rats did not reach the level of statistical significance whereas 12-day-old WAG/Rij pups exhibited significantly lower threshold for elicitation of stimulation-bound movements than the

two older groups. Limbic type of ADs was more easily elicited in 25-day-old than in 18-day-old rat pups in this strain. Movements bound to stimulation were most easily elicited in 18-day-old ACI animals whereas SW type of ADs as well as clonic seizures needed higher stimulation intensities in 12-day-old than in both 18- and 25-day-old rats. Surprisingly, transition to limbic type of ADs appeared in 12-day-old ACI rats at significantly lower intensities than in the two older groups but this type of seizures was elicited only in four animals.

If the three strains were compared (Fig. 2) 12-day-old rats exhibited a significant difference only in thresholds for limbic type of ADs; it was lower in ACI rats that in the two other strains. Eighteen- and 25-day-old WAG/Rij and ACI rats needed lower intensities to elicit SW type of ADs and accompanying clonic seizures than Wistar rats.

Twelve-day-old animals of all three strains generated longer ADs than older rats. The difference reached the level of statistical significance only with medium stimulation intensities in Wistar rats whereas it was significant with nearly all current intensities in the two inbred strains (Fig. 3). An enormous increase of duration of ADs with increasing intensities of stimulation observed in 12-day-old ACI rats (Fig. 3, uppermost part) was due to a transition to the limbic type of ADs at relatively low current intensities. Systematic significant differences between 18- and 25-day-old rats were found only in ACI rats—ADs in the younger group were longer than those in 25-day-old rats.

Comparison of ADs duration among the three strains did not show consistent changes. It demonstrated longer ADs in 12-day-old ACI rats than in the two other strains, some shorter ADs in 18-day-old WAG/Rij rats in comparison with ACI and Wistar rats and a few differences between 25-day-old Wistar and ACI rats (Fig. 3).

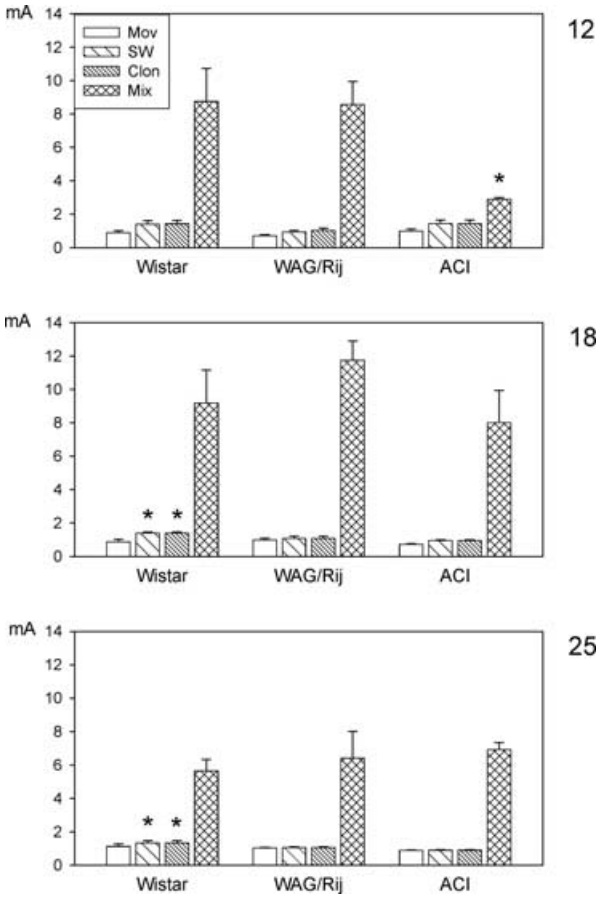

FIG. 2. Threshold intensities (mean + S.E.M.) for elicitation of stimulation-bound movements, spike-and-wave afterdischarges, clonic seizures, and limbic (mixed) type of afterdischarges (see inset) in 12-, 18-, and 25-day-old rats (from **top to bottom**). Abscissae from left to right: Wistar, WAG/Rij and ACI rats; ordinates: intensity of stimulation current in mA. Asterisks denote statistically significant difference in comparison with corresponding phenomenon in the other two strains.

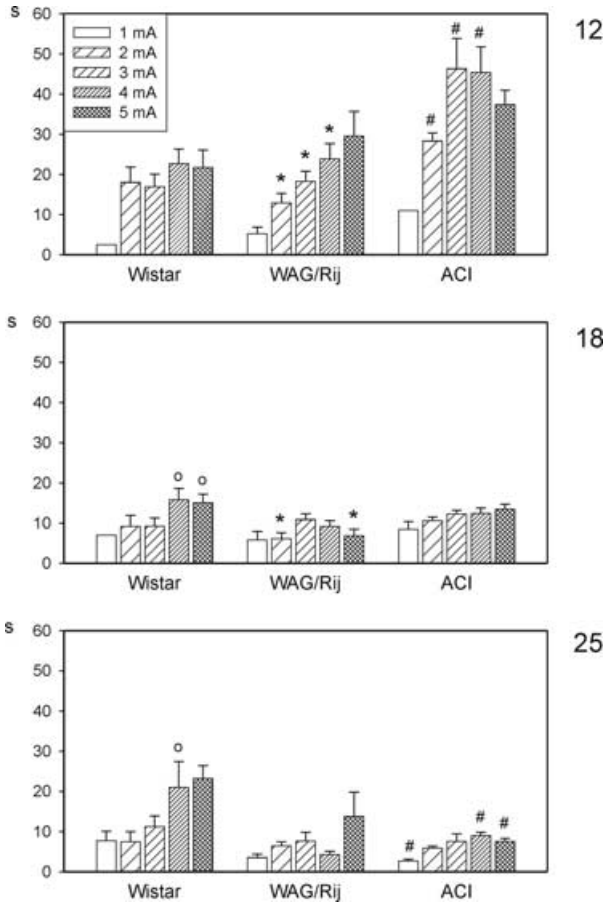

FIG. 3. Duration of afterdischarges (mean + S.E.M.) in 12-, 18-, and 25-day-old rats (from **top to bottom**). Individual columns—afterdischarges elicited by stimulation intensity of 1, 2, 3, 4, or 5 mA (see inset). Abscissae from left to right: Wistar, WAG/Rij, and ACI rats; ordinates: duration of afterdischarges in seconds. Circles mark a significant difference between Wistar and WAG/Rij rats, asterisks denote a difference between WAG/Rij and ACI rats, double crosses a difference between ACI and Wistar rats.

DISCUSSION

The difference in thresholds for SW and clonic seizures at the age 18 and 25 days was the same as in adult

animals—Wistar rats needed higher stimulation current intensities than WAG/Rij and ACI rats. The difference in cortical excitability between WAG/Rij and Wistar rats is in agreement also with data of D'Arcangelo et al. (2006). Unfortunately, these authors did not study ACI rats. No similar differences among the three strains were observed in 12-day-old pups—at the age when SW EEG rhythm cannot be elicited (Mareš et al., 1982; Schickerová et al., 1984; Snead, 1994). The only difference between adult WAG/Rij rats and two other strains (an easier transition to the limbic type of ADs) did not appear in our age groups. This transition matures during the fourth postnatal week in Wistar rats (Mareš et al., 1982) and therefore the difference may appear later in life.

Failure of changes specific for WAG/Rij rats could be due to the fact that SW episodes are present in all three strains and they differ only quantitatively (Inoue et al., 1990; Chocholová, 1983). Localization of stimulation electrodes might also play a role—they were localized more medially than the locus found by Meeren et al. (2002), but we used relatively large electrodes (surface in contact with dura mater was nearly 1×1 mm) so that the stimulated field is broad and at least at high stimulation intensities involves the locus of origin of SW episodes. The possible effect of stimulated area could be excluded only with cortical microstimulation. Nevertheless our results raise again an old question if an increased cortical excitability is the main change in generation of SW episodes. Higher excitability of cerebral cortex found in 18- and 25-day-old WAG/Rij and ACI pups in comparison with Wistar rats is in accordance with findings in pentetrazol- and gama-hydroxybutyrate-induced models of absences (Schickerová et al., 1984; Snead, 1994) as well as with electrically induced SW rhythm—it was recorded since the third postnatal week (Mareš, et al., 1982, 2002; Mareš and Kubová, 2006). This result is in contradiction with finding that SW episodes in the other strain with spontaneous absence seizures (GAERS) were recorded since the age of 40 days (Vergnes et al., 1986). Identical development of SW ADs in WAG/Rij and ACI rats indicate that generation of cortical epileptic ADs differs from generation of SW episodes. It is supported by a difference in frequency of SW activity induced by cortical stimulation and SW episodes (Mareš et al., 1982). Further analysis of this possibility including detailed ontogenetic study of spontaneous EEG activity of WAG/Rij rats is necessary.

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