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The effects of callosal agenesis on the susceptibility to seizures elicited by pentylenetetrazol in BALB/cCF mice

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Abstract

The effects of callosal agenesis in sensitivity to pentylenetetrazol (PTZ) were studied in 199 (95 males and 104 females) mice of the BALB/cCF strain. This strain presents agenesis of the corpus callosum (CC) in approximately 30% of its population. Seizures were elicited by intraperitoneally injected PTZ. Animals were tested with doses of 40 and 50 mg/kg. Seizure severity was expressed by the following scoring scale: 0 (*no abnormal behavior, NAB*); 1 (*myoclonus, M*); 2 (*running bouncing clonus, RBC*); 3 (*tonic hindlimb extension, THE*). For the 40-mg/kg dose, abnormal mice were found to be more susceptible, displaying more severe seizures more often then normal mice. Normal female mice were also more susceptible to PTZ than males for this dose. No significant differences were found for the 50-mg/kg dose as a result of the fact that most animals displayed RBC. These data indicate that callosal development and sex are important factors affecting seizure susceptibility. © 2002 Elsevier Science Inc. All rights reserved.

Keywords: Callosal agenesis; Sex differences; Pentylenetetrazol (PTZ); γ-Aminobutiric acid (GABA); Seizures; Mice

1. Introduction

The corpus callosum (CC) is the largest interhemispheric cerebral commissure in eutherian mammals (Ebner and Myers, 1965). One of its main functions at adulthood is that of integrating the two mental domains emerging from the cerebral hemispheres by constantly transferring information between them (Lent and Schmidt, 1993). An untoward consequence of this integrative capability is that the CC is also responsible, in some cases, for spreading the epileptic activity from one hemisphere to the other (Alefeld et al., 1998). Interestingly, the agenesis of this commissure also seems to have a deleterious effect regarding epilepsy. For instance, a higher incidence of epileptic seizures is noticed in humans presenting the Aicardi syndrome (Aicardi and Chevrie, 1994). In such cases, normal brain development was disrupted, resulting, among other malformations, in callosal agenesis.

Identifying the roles played by the CC, either during development or later in adult life, frequently involves the use of animal models of early callosal absence (Lent and Schmidt, 1986; Schmidt, 1994; Schmidt and Caparelli-Dáquer, 1989; Schmidt et al., 1991). For instance, Dolina et al. (1993) investigated the influence of callosal agenesis on epilepsy in rodents. Using mice of the BALB/c strain, these researchers did a selective breeding based on susceptibility to audiogenic seizures (Dolina et al., 1993). This breeding process resulted in two substrains: one that was epilepsy-prone (EP) and another that was epilepsy-resistant (ER). Subsequently, Morin et al. (1994) observed that all EP mice presented with callosal agenesis, while all ER mice had a normal CC. However, considering that the two substrains were bred for audiogenic seizure susceptibility, one may suggest that the higher sensitivity in one of the substrains cannot be conclusively attributed to the developmental consequences of the agenesis of the CC.

In the inbred BALB/cCF mice strain, callosal agenesis occurs in approximately 30% of the population (Wahlsten,

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1987; Schmidt and Caparelli-Dáquer, 1989; Schmidt et al., 1991). In the same litter, it is possible to find acallosal and normal animals. In this sense, this strain has a control group that is virtually indistinguishable from the acallosal group until after anatomical/histological procedures are carried out. In the present work, we tested our hypothesis that acallosal mice are more susceptible to seizures than normal mice by measuring sensitivity to seizures induced by pentylenetetrazol (PTZ, which is one of the most frequently used convulsants) in BALB/cCF mice. Furthermore, taking into account that PTZ is a noncompetitive GABA antagonist (Olsen, 1981; Macdonald and Olsen, 1994) and that it has been demonstrated that sexual hormones can modulate the activity of the GABA receptor complex (Wilson, 1992), thereby altering brain excitability, we also investigated the effect of sex on the susceptibility to seizures induced by PTZ.

2. Method

2.1. Subjects

We used 199 adult BALB/cCF mice (95 males and 104 females). This substrain was established by Carworth Farms from pedigreed BALB/c mice obtained in 1968 from the Laboratory Animal Center in England. Original breeding stock was kindly provided by Dr. Douglas Wahlsten (University of Alberta, Edmonton, Alberta, Canada) and approximately 27 generations have been bred in our laboratory. Animals were maintained on a 12:12-h light/dark cycle (2:00 a.m.–2:00 p.m. light period), being housed at a constant temperature (22 °C) and having unrestricted access to food and water. They were not habituated to intraperitoneal injection of PTZ before the beginning of the experiments. The experimental sessions were carried out between 8:30 a.m. and 12:30 p.m. in a noise-free room and both sexes were always used in the same session. Each animal was tested only once. On average, 6.6 ± 0.3 (mean ± S.E.M.) animals were tested per day.

2.2. Test procedure

PTZ (free base—Sigma) freshly dissolved in 0.9% NaCl solution was administered intraperitonealy in doses of 40 mg/kg ($n = 98$) or 50 mg/kg ($n = 101$). We choose the 50-mg/kg dose because this dose is considered the CD_{50} for the mouse (Stone, 1970). Since we expected acallosal mice to be more susceptible to PTZ, we also used the 40-mg/kg dose. Immediately after injection, the animals were individually placed in a glass box ($12 \times 24 \times 17$ cm), which had a transparent front wall. The behavior displayed by each animal was then recorded for 10 min. These recordings were subsequently used to evaluate the severity of the seizures.

Seizure severity was expressed by the following scoring scale (Finn and Gee, 1994; Medina et al., 2001):

1. No abnormal behavior (NAB);
2. Myoclonic twitches (M—sudden muscle jerk, sometimes accompanied by tail movements and head twitch);
3. Running bouncing clonus (RBC—violent whole body clonus, with or without loosing of righting reflexes);
4. Tonic hind limb extension (THE—extreme rigidity with fore- and hindlimbs extended caudally).

Each animal received a final score that corresponded to the most severe seizure it presented during the test.

2.3. Histology and diagnostic procedures

After the tests, all mice were deeply anaesthetized with ether and perfused through the heart with saline followed by a solution of paraformaldehyde (4%). The heads were stored in the same solution for at least 7 days. After this postfixation time, the brains were removed. The hemispheres were separated along the midsagittal plane and stained with golden chloride (Schmued, 1990). As a result of the staining procedure, the interhemispheric commissures (including the CC) became dark brown in color, which contrasted with the light background.

For each sex, the normal group was composed of animals whose callosal lengths were greater than or equal to the respective medians. To determine those with abnormal CC, a 99.9% confidence limit was set around the medians. Mice with callosal lengths below that limit were considered abnormal. The remaining mice were defined as borderline (all animals from this group were eliminated form the behavioral analysis). It should be mentioned that the data of callosal lengths were very skewed. Supposing that extremely low values would not affect the median, less than 5% of borderline animals would have been erroneously considered as abnormal.

2.4. Statistics

Initially, Kendall's coefficient of rank correlation (τ) was calculated in order to determine if seizure scores were significantly affected by absence of CC, sex, and dose. Subsequently, statistical analysis of the scores was performed separately on the data from each dose group. The average scores for normal and abnormal groups and male and female groups were calculated (results are presented in terms of means ± S.E.M.). Differences in the average scores between groups were evaluated by means of Mann–Whitney U tests (M-W). Differences between groups in the percentage of animals presenting a given score were compared by means of chi-square tests. The effects of absence of CC, dose, and sex on latencies were evaluated by using a general factorial ANOVA. Differences between the means

were assessed by Student's t test. Results were considered significantly different at $P < .05$.

3. Results

3.1. Morphological analysis (Fig. 1)

For the male mice, the normal group was composed of animals whose callosal lengths were equal to or greater than 3.43 mm ($n = 48$) (Fig. 1). The abnormal group was composed of animals whose callosal lengths were equal to or smaller than 2.88 mm ($n = 32$). Those mice with callosal lengths between 2.88 and 3.43 mm were included in the borderline group ($n = 15$). For the female mice, the normal group was composed of animals whose callosal lengths were equal to or greater than 3.65 mm ($n = 51$). The abnormal group was composed of animals whose callosal lengths were equal to or smaller than 2.65 mm ($n = 35$). Mice with callosal lengths between 2.65 and 3.65 mm were included in the borderline group ($n = 18$).

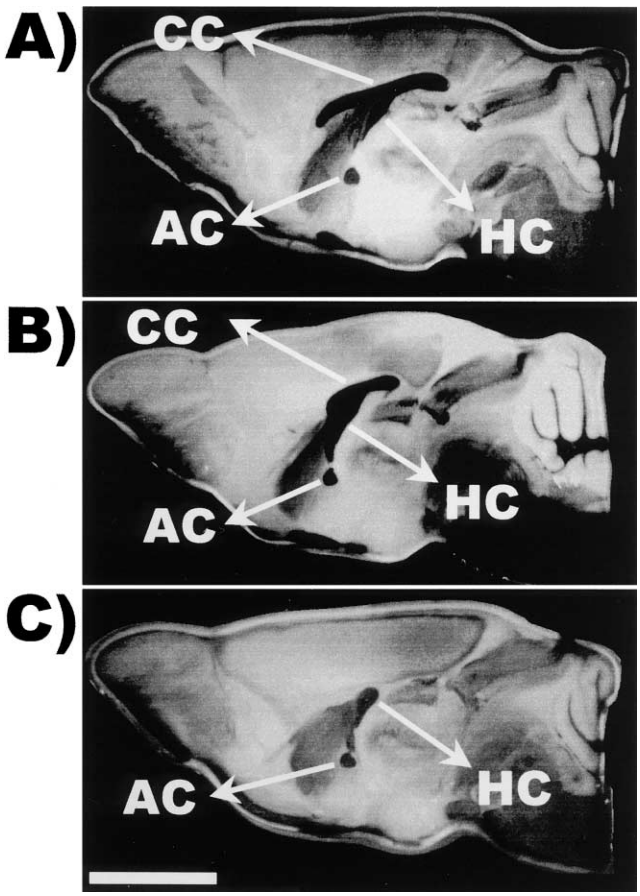

Fig. 1. Midsagittal views of golden-chloride-stained hemispheres of adult BALB/cCF mice. (A) Normal CC. Note that all interhemispheric commissures were darkened by the staining procedure. (B) Partial agenesis of the CC. (C) Total agenesis of the CC. CC: corpus callosum; AC: anterior commissure; HC: hippocampal commissure. Calibration = 3 mm.

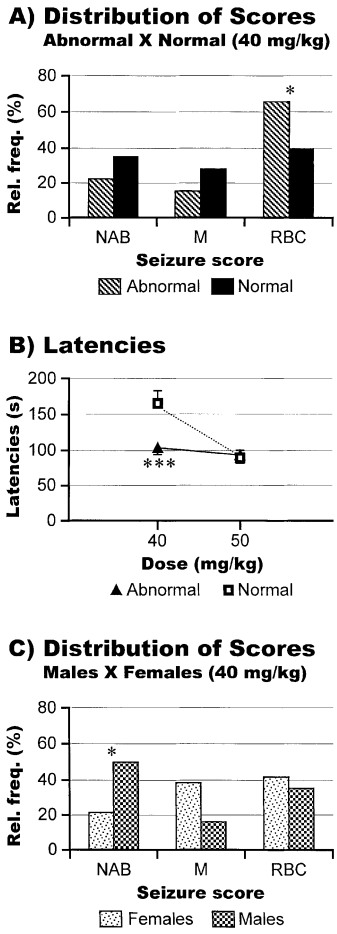

Fig. 2. (A) Distribution of scores according to presence/absence of the CC for the 40-mg/kg dose. Note that the percentage of abnormal mice presenting RBC is significantly higher than that of the normal ones. This result indicates that abnormal mice are more susceptible to PTZ (ip)-induced seizures than normal mice. NAB: no abnormal behavior; M: myoclonus; RBC: running bouncing clonus. (B) Dose–response curve showing the latencies for the onset of the first observed seizure (regardless of severity). Note that latency decreases when the dosage is increased. A significant difference exists for the 40-mg/kg dose between abnormal and normal mice, indicating that the former group is affected by the PTZ faster than the latter. Each point represents the mean ± S.E.M. (C) Distribution of scores according to sex group. Note that the percentage of males that did not display abnormal behaviors is significantly higher than that of females for the 40-mg/kg dose. This result indicates that female mice are more susceptible to PTZ (ip)-induced seizures than male mice. NAB: no abnormal behavior; M: myoclonus; RBC: running bouncing clonus. *** $P < .001$; ** $P < .01$; * $P < .05$.

3.2. Behavioral analysis

Significant correlations between absence of CC and score ($\tau = .216$; $P < .01$) and dose and score ($\tau = .409$; $P < .001$) were demonstrated. The correlation between absence of CC and score can be explained by the higher mean score of the abnormal group (1.43 ± 0.16) as

compared to the normal group (1.0 ± 0.11) when the 40-mg/kg dose was used (M-W; $Z = 2.01$; $P < .05$). In fact, the distribution of scores demonstrated that the abnormal group displayed the more severe RBC seizure more often than the normal group (Fig. 2A). The correlation between dose and score can be explained by the fact that the 50-mg/kg dose resulted in more severe seizures more often than the 40-mg/kg dose. In fact, the 50-mg/kg dose resulted in a high occurrence of RBC in both groups (abnormal = 92%; normal = 87%). This difference between groups was not statistically significant.

Regarding latencies to the first seizure, the ANOVA revealed an effect of absence of CC ($F = 4.602$; $P < .05$), dose ($F = 10.1$; $P < .01$) and an interaction of absence of CC and dose ($F = 5.824$; $P < .05$). These effects could be explained by the fact that, with the 40-mg/kg dose, the acallosal group presented a mean latency ($\bar{X} = 102.7$ s, S.E.M. = 8.6) that was significantly lower ($t = 3.09$; $df = 50$; $P < .001$) than that displayed by the normal group ($\bar{X} = 161.4$ s, S.E.M. = 16.9) (Fig. 2B).

No significant differences regarding average scores and mean latencies were observed between the normal and the abnormal groups when the 50-mg/kg dose was used.

In order to avoid any interaction between callosal agenesis and sex, only normal animals were used to study the effect of sex in the susceptibility to PTZ-induced seizures. For the 40-mg/kg dose, the average scores did not show a significant difference between sexes (M-W; $Z = 1.5$; $P = .13$). However, while the shape of the scoring distribution of the female group indicated a progressive increase in the number of animals toward higher scores (6 had NAB, 11 had M, and 12 presented with RBC), the male distribution appeared to be U-shaped (13 had NAB, 4 had M, and 9 presented with RBC). In fact, significantly less ($\chi^2 = 5.2$; $df = 1$; $P < .05$) females displayed NAB than males (20.7% and 50.0%, respectively) (Fig. 2C). Administration of 50 mg/kg resulted in similar distributions of scores for both males and females (data not shown).

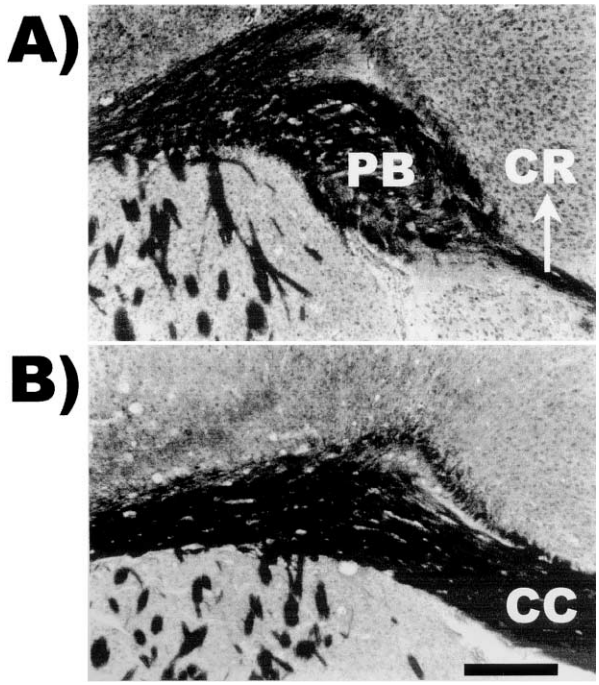

Fig. 3. Photomicrographs of golden-chloride-stained coronal sections of an abnormal mice (A) as compared to that of a normal mice (B) at the level of the anterior commissure. (A) Note the tortuous and convoluted course of the fibers that make up the Probst's longitudinal bundle. Also present is a small callosal remnant that approaches the midline between the hemispheres. CC: normal corpus callosum; CR: callosal remnant; PB: Probst's bundle. Calibration = 100 μm.

4. Discussion

The results obtained with the 40-mg/kg dose showed that abnormal mice are more susceptible to seizures than normal ones. It was also demonstrated that normal female mice are more susceptible than males. Our results indicated that the 50-mg/kg dose of PTZ is not the CD_{50} for the BALB/cCF strain of mice since in between 87% and 92% of them (depending on presence/absence of the CC) displayed RBC.

The absence of the CC is frequently accompanied by the presence of aberrant fibers bundles (Schmidt and Caparelli-Dáquer, 1989). Morin et al. (1994) suggested that these fibers are, indeed, functional. In this sense, animal models of callosal agenesis could be rendered more susceptible to epileptic seizures due to the activity of these fibers. In fact, abnormal BALB/cCF mice present a remarkable aberrant fiber bundle, known as the Probst's longitudinal bundle, in both hemispheres (Fig. 3). This medially located anomalous fiber agglomerate runs rostrocaudally underneath the white matter, being particularly prominent in more anterior levels (Silver et al., 1982). Several authors indicated that the Probst's bundle is formed by fibers that would have constituted the CC but failed to cross to the contralateral hemisphere (Silver et al., 1982; Ozaki et al., 1987, 1989) due to the delayed formation, malformation, or absence of a transitory layer of subventricular glial cells (glial sling) extending form the lateral ventricle to the midline (Wahlsten, 1987; Ozaki and Wahlsten, 1992, 1993) that would act as an intermediate guidepost for developing callosal axons (Shu and Richards, 2001). Thus, callosal agenesis in this model seems to be the result of a disruption of the normal pattern of development that is restricted to a specific region of the telencephalic midline (Wahlsten, 1987; Ozaki and Wahlsten, 1993). However, some of the fibers of the Probst bundle seem to present significant ipsilateral homotopic projections (Ozaki et al., 1987, 1989). In this sense, it is conceivable to speculate that these projections could be acting as a reverberating system, rendering abnormal mice more susceptible to seizures. In effect, cortical reverberating systems have already been implicated in epileptogenesis (Rutecki et al., 1989; Stringer and Lothman, 1992). On the other hand, another possible explanation for the higher

susceptibility of abnormal mice could be related the inhibitory role of CC. In spite of the frequent use of callosotomy in intractable epilepsy (Mamelak et al., 1993), it has been observed that, in some cases, partial seizures are enhanced by this procedure (Spencer et al., 1984, 1988). This increase in brain excitability has been attributed to the lack of the inhibitory action of CC (Spencer et al., 1984, 1988). However, PTZ is used as a model of generalized seizures. In this sense, a direct link between the inhibitory role of the CC and increased brain excitability in such model remains to be demonstrated.

The differences between abnormal and normal mice observed in our study are not as striking as those found by Dolina et al. (1993) between ER and EP BALB/c mice (ER mice presented a threshold for intramuscularly injected PTZ of 50 mg/kg while ER mice had a threshold of 25–30 mg/kg). Taken together, these findings suggest that factors other than (or in addition to) the agenesis of the CC could explain those great differences in susceptibility to seizures found in the Dolina et al. study. Considering that it has been shown that several genes are related to epilepsy (Puranam and McNamara, 1999), the selective breeding performed by Dolina et al. based on susceptibility to audiogenic seizures did not exclude the possibility that the absence of the CC in the EP mice was an associated factor not directly related to their susceptibility. In the present study, both normal and abnormal BALB/cCF mice are found in the same litter. Considering that the BALB/cCF is a highly inbred strain of mice, normal and abnormal mice share the same genetic background. Therefore, in this strain, the higher susceptibility of abnormal mice can be more directly linked to the absence of the CC.

Regarding the effect of sex, in a previous study we demonstrated significant differences in the sensitivity to intraperitoneally injected PTZ between male and female Swiss mice, females being more susceptible than males (Medina et al., 2001). Our results in normal BALB/cCF corroborate this previous finding by demonstrating significant differences between sexes in the percentage of animals that did not present seizures.

In conclusion, our results confirm and extend previous studies indicating that callosal agenesis in mice is associated with a higher susceptibility to seizures and that gender is a critical factor influencing susceptibility to PTZ-elicited seizures.

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