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Persistence of Acquired Epileptogenesis in Amygdaloid-Kindled Rats: Relationship between the Initial Kindling Stages and Seizure Development in Rekindling

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In order to gain insight into mechanisms underlying the persistence of the partial kindling effect, we suspended amygdaloid kindling at different seizure stages in rats, and investigated the effects on subsequent rekindling after a rest period of 2 months. Ten-week-old rats, implanted with bipolar electrodes, were separated by various initial kindling stages into 5 groups of rats, partially kindled to stages 1 (n = 7) and 2 (n = 10), fully kindled to stages 4 (n = 11) and 5 (n = 11), and control rats (n = 12) which were implanted but not stimulated initially. The number of stimulations required to elicit the first stage 5 seizure during rekindling was significantly lower in the fully kindled groups ($P < 0.01$ in the stage 4 group and $P < 0.001$ in the stage 5 group) than the control group. The cumulative afterdischarge duration during rekindling was significantly shorter in the fully kindled groups ($P < 0.01$ in both groups) than the control group. The latency of the first stage 5 seizure during rekindling was significantly shorter in the partially and fully kindled groups than the control group. These results suggest that epileptogenesis acquired at the partially kindled stage is different than that acquired at the fully kindled stage. However, the effects of the initial kindling on the latency to produce the stage 5 seizure during rekindling persisted both in the partially and fully kindled rats.

Key words: amygdaloid kindling; epileptogenesis; partial seizure; rat; rekindling

One potential reason for the intractability of epileptic seizures has been reported as symptomatic localized epilepsy with complex partial and secondarily generalized seizures (Okuma and Kumashiro, 1981) as well as a high incidence of seizures before the start of treatment (Reynolds, 1990). In addition, the possible role of the long-term persistence of epileptogenesis continues to be debated.

Limbic kindling is a well-known model of temporal lobe epilepsy (Sato et al., 1990), with a long-term persistence of acquired susceptibility (Goddard et al., 1969; Wada et al., 1974; Wada, 1980; Sato et al., 1990). As for the degree of kindling relationship between the seizure stage before the completion of kindling and the persistence of its effect, Moshé and Albala (1982) reported that the kindling effect persisted into adulthood when the previously fully and partially kindled pups were rekindled at maturity. Similarly, Dennison et al. (1995) reported that a partial kindling effect was retained during a 12-week period in adult rats. However, it is not clear at what stage of partial kindling the effect becomes persistent. In the present amygdaloid kindling study, therefore, we investigated the effects of various stages of initial kindling on the pattern of subsequent rekindling following a rest period of 2 months.

Abbreviation: AD, afterdischarge

Materials and Methods

Animals

Fifty-one 10-week-old male Wistar rats were used. The rats were randomly divided into an experimental group (n = 39) and control group (n = 12), maintained on lab chow and water ad libitum in an environmentally controlled room (12/12 h light/dark cycle, lights on at 0700, temperature 20–22˚C, humidity 30–40%) in the Laboratory Animal Research Center, Faculty of Medicine, Tottori University. All experiments conformed to the Guidelines for Animal Experimentation at the Faculty of Medicine, Tottori University.

Surgery

Under pentobarbital anesthesia (intraperitoneal administration of 40-mg/kg Nembutal; Abott, Chicago, IL), stimulating and recording stainless-steel bipolar electrodes 200 μm in diameter were stereotaxically (König and Klippel, 1970) implanted into the right amygdala (P = 3.0 mm, L = 4.0 mm and H = 8.8 mm relative to the bregma) and a reference electrode was placed on the skull over the frontal bone. After a 1-week postoperative recovery period, the kindling study was started.

Kindling procedures

Kindling was begun with 60-Hz sinusoidal electrical stimulations applied to the right amygdala at the afterdischarge (AD) threshold for 1 s once every day. The AD threshold was determined by increasing the stimulation intensity by 50 μA every 15 min from the initial intensity of 100 μA until an AD was induced. Electrographic recording was made throughout to measure the duration of ADs, which were correlated with behavioral seizure stages. The behavioral seizure stages of amygdaloid kindling were assessed with the classification proposed by Racine (1972): stage 0, AD but no convulsions; stage 1, mouth and facial movement; stage 2, head nodding; stage 3, forelimb clonus; stage 4, rearing; stage 5, rearing and falling. Stages 1 and 2 are thought to correspond to the partial seizure stages, and stages 4 and 5, to the secondarily generalized seizure stages.

Initial kindling and rekindling

The control rats (n = 12) were implanted with electrodes but not stimulated for 2 months until the time of rekindling. The experimental group (n = 39) was initially kindled according to the above-described procedures, and divided into the following 4 subgroups: Stage-1 (n = 7), Stage-2 (n = 10), Stage-4 (n = 11) and Stage-5 (n = 11). Stimulation was suspended after a stage 1 seizure developed in the Stage-1 group, and similarly after a seizure of stages 2, 4 and 5 developed in the Stage-2, -4 and -5 groups, respectively. The rats of the Stage-1 and Stage-2 groups were arbitrarily categorized as partially kindled, and those of the Stage-4 and Stage-5 groups, as fully kindled.

Two months later, the animals were rekindled at the AD threshold which was determined with the same method as in the initial kindling. The rats received electrical stimulation at the maximum AD threshold for each group once a day till a stage 5 seizure was induced for 5 consecutive days. Behavioral seizure stages during rekindling and the duration of ADs after each rekindling stimulation were recorded and evaluated with the same method as in the initial kindling.

Data analysis

The number of electrical stimulations that elicited the first seizure in stages 1 to 4 in the initial kindling was recorded in each rat from the 4 experimental groups. The number of electrical stimulations used in one of the seizure stages to elicit the first stage 5 seizure during rekindling was recorded in each rat of the control and 4 experimental groups.

Furthermore, we totaled up the duration of ADs at the manifestation of respective seizure stages during rekindling in the control and 4 experimental groups. Peterson et al. (1981)

reported that the cumulative AD duration was a more reliable indicator in the development of kindling than the actual number of stimulations required to elicit ADs. So, we calculated the AD duration cumulated in one seizure stage and the cumulative AD duration totaled up from the first stimulation until the completion of kindling, and compared the obtained results among the control and experimental groups.

The latency of the onset of generalized seizures, i.e., the time needed to produce a stage 5 seizure after the start of stimulation, was recorded for 5 consecutive days during rekindling in the control and 4 experimental groups. Data were expressed as means ± SD. Statistical analysis was carried out with Mann-Whitney's *U*-test or Student's *t*-test.

Results

Number of stimulations in initial kindling and rekindling

Table 1 shows the number of stimulations needed to elicit the first seizure of each stage in the initial kindling and used in each seizure stage during rekindling. In the Stage-5 group, the number of stimulations used to develop the first stage 5 seizure in rekindling was significantly lower than that in the control group. In addition, the number of stimulations totaled up from stages 0 to 4 during rekindling was also significantly lower than that in the control group; in fact, the number of stimulations from stages 1 to 4 was zero in the Stage-5 group because this group directly progressed to stage 5 in rekindling. In the Stage-4 group, the number of stimulations needed to develop the first stage 5 seizure in rekindling was also significantly lower than that in the control group. The number of stimulations needed in stages 0 and 3 in rekindling was also significantly lower than that in the control group. In the Stage-1 and Stage-2 groups in which the initial kindling was suspended at the partial seizure stage, the number of stimulations that elicited the first stage 5 seizure in rekindling was not significantly different from that in the control group in rekindling. On the other hand in the Stage-2 group, the number of stimulations needed in stages 0 and 3 in rekindling was significantly lower than that in the control group. No significant differences were seen between the Stage-1 and control groups in the number of stimulations needed in each seizure stage in rekindling.

Cumulative AD duration in rekindling

The means of AD duration cumulated in each seizure stage during rekindling classified by groups are shown in Table 2. In both the Stage-4 and Stage-5 groups, the cumulative AD duration totaled up from stages 0 to 4 was significantly shorter than that in the control group.

Table 1. The effects of initial kindling stages on seizure manifestations during rekindling

Group	Number of stimulations						
	Initial kindling†	Rekindling‡					
		Stage 0	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
Control [12]	—	2.9 ± 2.0	1.4 ± 1.8	0.5 ± 0.5	0.5 ± 0.5	0.7 ± 0.8	7.0 ± 3.9
Stage-1 [7]	4.1 ± 1.9	3.3 ± 4.5	2.3 ± 3.0	1.0 ± 1.4	0.1 ± 0.4	1.3 ± 1.4	9.0 ± 6.5
Stage-2 [10]	9.4 ± 3.5	1.2 ± 1.6*	0.4 ± 0.7	0.9 ± 0.7	0.1 ± 0.3*	0.7 ± 0.8	4.3 ± 1.5
Stage-4 [11]	10.6 ± 3.7	0.3 ± 0.7***	0.3 ± 0.7	0.4 ± 0.5	0.1 ± 0.3*	0.5 ± 0.7	2.5 ± 1.0**
Stage-5 [11]	9.6 ± 4.8	0.2 ± 0.6***	0*	0**	0**	0**	1.2 ± 0.6***

Mean ± SD.
[ ], number of animals used.
† Number of stimulations that elicited the first seizures of stage 0 to stage 5 in the initial kindling.
‡ Number of stimulations used in one of the seizure stages or required to elicit the first stage 5 seizure during rekindling.
* $P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with the control group (Mann-Whitney's *U*-test).

Table 2. The effect of initial kindling stages on the cumulative AD duration needed to elicit the first stage 5 seizure during rekindling

Group	Cumulative AD duration (s)					
	Stage 0	Stage 1	Stage 2	Stage 3	Stage 4	Total
Control	186.2 ± 217.4	107.1 ± 156.1	44.3 ± 54.5	50.5 ± 59.6	48.8 ± 65.1	436.8 ± 403.2
Stage-1	191.0 ± 387.5	149.0 ± 247.4	50.1 ± 68.5	15.6 ± 41.2	93.1 ± 96.0	498.9 ± 554.7
Stage-2	33.9 ± 52.8*	20.6 ± 46.5	65.5 ± 66.9	2.2 ± 7.0*	64.7 ± 86.9	186.9 ± 114.1
Stage-4	3.6 ± 9.2*	15.1 ± 33.7	11.6 ± 18.9	3.6 ± 11.8*	43.0 ± 81.1	76.8 ± 72.7**
Stage-5	3.5 ± 11.5*	0*	0*	0*	0*	3.5 ± 11.5**

AD, afterdischarge.
* $P < 0.05$, ** $P < 0.01$ compared with the control group (unpaired Student's *t*-test).

However, in the Stage-5 group, the cumulative AD duration in stages 1 to 4 was zero due to the direct progression to stage 5 in rekindling. No significant differences were seen between the Stage-1 and control groups in the cumulative AD duration totaled up in all seizure stages during rekindling.

Latency of the onset of generalized seizures in rekindling

Figure 1 shows the mean values of latency of the first stage 5 seizure during rekindling classified by groups. The mean latencies were significantly shorter in all 4 experimental groups than in the control group. In the Stage-5 group, the mean latencies were significantly shorter than those in the control group, even when the stage 5 seizures occurred on days 2 and 3, but not significant in the 3 other experimental groups other than in the control group. On days 4 and 5 also, the differences in mean latency of the first stage 5 seizures during rekindling were not significant between the control group and any of the 4 experimental groups.

Discussion

In the present study, the previously partially and fully kindled rats were rekindled 2 months later, and the effects of differences in seizure stages during the initial kindling in seizure manifestations during rekindling were investigated. The fully kindled groups showed significantly lower levels in the number of stimulations required to elicit the first stage 5 seizure during rekindling than the control group did. The difference was not significant between the partially kindled groups and the control group. In other words, susceptibility acquired at the partial seizure stage was different from that

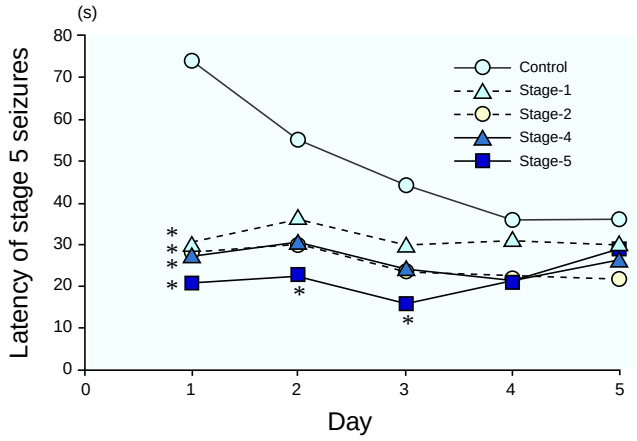

Fig. 1. The latencies needed to elicit a stage 5 seizure for 5 consecutive days during rekindling. Symbols are means of the latencies. *$P < 0.05$ compared with the control group (Student's *t*-test). On day 1, the latencies in the Stage-1, -2, -4 and -5 groups are significantly shorter than in the control group. On days 2 and 3, only the Stage-5 group shows a significantly shorter mean than the control group.

acquired at the secondarily generalized seizure stage, and only the latter persisted to make an impact on rekindling carried out 2 months later. This finding is consistent with the differential effects observed in cumulative AD duration (Table 2).

On the other hand, the latency of the first stage 5 seizure during rekindling was significantly shorter in both the partially and fully kindled groups than in the control group. This finding conflicted with the results that the difference in number of stimulations and cumulative AD duration during rekindling was not significant between the partially kindled and control rats. The reason why the latency of the first stage 5 seizure during rekindling was shortened even in the partially kindled rats is difficult to explain from the results of the present experiments only. However, our results clearly showed that the latencies on days 2 and 3 were significantly shorter only in the Stage-5 group than in the control group. The kindling effects in the Stage-5 group were stronger and more persistent than in the other experimental groups.

Dennison et al. (1995) carried out kindling experiments in rats which reached stages 1, 3 and 5, and rekindled 12 weeks after kindling suspension. They compared the number of stimulations that elicited generalized seizures in the 3 experimental and control groups, and found that the number of stimulations that elicited generalized seizures was significantly lower in both the Stage-3 and Stage-5 groups than in the control group. The difference was not significant between the Stage-1 group and the control group. This Stage-1 group had onsets of seizures even after the first stimulation during rekindling. Hence, they concluded that the effects of kindling were persistent without further stimulation, and were also retained to some degree even in the group partially kindled to stage 1. Moshé and Albala (1982) performed amygdaloid kindling in developing rats: in both fully and partially kindled rats, the kindling rate during rekindling in the same, adult rats was significantly lower than in the control rats. Thus, kindling-induced neurobiological changes in developing rats persisted into adulthood even in the partially kindled. However, the AD duration of the first seizure and the AD duration of the first generalized seizure during rekindling were only significantly longer in the fully kindled group than in the control group. Therefore, they concluded that the degree of retention of the kindling effects was stronger in the fully kindled group than in the partially kindled group. On the other hand, since both the AD duration and seizure stage were retained during rekindling in rats that had been kindled to stages 1 to 4 and then left unstimulated for 45 days, regression of the kindling effect is not dependent on the seizure stage prior to interruption of stimulation (Homan and Goodman,1988). In the present experiments, both the number of stimulations that elicited the first stage 5 seizure and the AD duration during rekindling in the partially kindled group, unlike in the fully kindled group, were not significantly different from those in the control group. However, the initial kindling exerted an effect on the latency of the first stage 5 seizure during rekindling, even in the partially kindled group. Hence, a further detailed investigation is needed to clarify whether a long-term persistence of epileptogenesis is acquired in partial kindling or not.

As for the anatomic relationship between amygdaloid kindling and the seizure development stage following kindling stimulation, it is thought that a functional connection is formed between the amygdala and the cerebral cortex of the motor area on the stimulation side in the case of progression from partial seizure stages to stage 3 (Wada, 1982). In addition, a functional connection is thought to be formed among the cortical and subcortical nuclei in both hemispheres in monkeys, especially in the bilateral motor areas, when stage 3 progresses to stage 4 and the seizures become secondarily generalized (Baba et al., 1986). Burchfiel and Applegate (1989) hypothesized, on the basis of their results using a kindling antagonism model, that seizure development in kindling is not a continuous process but one that follows a stepwise progression. In their view, stages 1 and 2 are the stages of transient neural reorganization in which the forebrain gate is opened, and stage

3 involves permanent neural reorganization in which the brain stem gate is opened, leading to the generalized seizures of stages 4 and 5. Therefore, the differences in the degree of retention of kindling effects between partially and fully kindled groups in the present study may represent differences in the degree of functional connection between the forebrain and the brain stem.

It is thought that amygdaloid kindling induces morphometric changes in the nervous system, such as mossy fiber sprouting (Cavazos and Sutula, 1990) and neurogenesis (Scott et al., 1998) in the dentate gyrus of the hippocampus, leading to synaptic reorganization and the development of epileptogenesis. However, Osawa et al. (1995) reported that the degrees of neural sprouting in the mossy fiber of the dentate gyrus of the hippocampus of amygdala-kindled rats which had reached stage 5 and those that had only reached stage 3 were not significantly different as compared with the degree in the control rats. Thus, they concluded that their neuropathological findings were not sufficient to explain kindled seizure susceptibility by neural sprouting in the dentate gyrus of the hippocampus. Uemura et al. (1996) performed rekindling following a rest period of 2 weeks after the initial kindling in perforant path-kindled rats, but they found no correlations between the degree of mossy fiber sprouting and the AD duration or seizure stage during rekindling. Therefore, they concluded that mossy fiber sprouting has no relationship to kindling development. Recently, Scott et al. (1998) reported that granular cells were newly formed in the dentate gyrus of the hippocampus of rats which had reached stage 5 by amygdaloid kindling. However, they found that there was no new growth of neural cells in rats which showed only localized seizure manifestations after several kindling stimulations. Hence, they concluded that the stage of seizure elicited by kindling and the degree of new neural cell growth are closely related. Causes of the differential effects of partial and full kindling remain to be elucidated. Evaluation of a possible relationship between morphometric changes mentioned above and the differential effects of kindling described in the present study may shed some light on this question.

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