Suppressive effect of callosotomy on epileptic seizures is due to the blockade of enhancement of cortical reactivity by transcallosal volleys.

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Abstract

The present work demonstrates that cortical reactivity of the rat, monitored by thalamocortical responses, can be enhanced by repetitive transcallosal volleys (5-20 Hz). This effect can be completely inhibited by callosotomy. We believe that the interhemispheric epileptogenesis and the suppressive effect of callosotomy for intractable epilepsy can be explained by this facilitatory effect of the corpus callosum on the cerebral cortex.

Keywords: Corpus callosum; Cortical reactivity; Facilitation; Epilepsy; Callosotomy; Rat

Introduction

Callosotomy is a surgical option for intractable epilepsy. It is generally accepted that the effect of this procedure is to prevent the propagation and the synchronization of seizure discharges (Gates et al., 1984; Spencer 1988). EEG changes after callosotomy also partly support this idea, i.e., preoperatively observed bilateral discharges turned into lateralized or asynchronized ones postoperatively (Gates et al., 1984; Spencer 1988). In many cases, however, callosotomy decreases seizure frequency and severity rather than transforming the seizure pattern from a generalized form into a partial one (Gates et al., 1987; Baba et al., 1996) and marked reduction or complete disappearance of seizure discharges on EEG recordings has been obtained in some patients (Gates et al., 1984; Spencer, 1988; Baba et al., 1996). These observations indicate that the callosotomy not only interrupts the seizure propagation but also suppresses the cortical epileptogenisity itself. Clinically, EEGs of patients who submitted to callosotomy generally show bilateral bursts of seizure discharges. In such situations, "massive" transcallosal volleys seem to run concurrently with EEG burst discharges. If transcallosal volleys facilitate cortical reactivity, the corpus callosum functions as a positive feedback loop, and thus by disconnecting the loop, callosotomy can suppress the volleys, resulting in reduction of seizure discharges. In this context, cortical reactivity under conditioning stimuli applied to the corpus callosum (at various frequencies mimicking bursting seizure discharges) was evaluated using rats.

Methods

Male Wistar rats (8-12 weeks, 300-450 g) were used in this study. They were anesthetized by urethane (1.2g/kg, i.p.) and placed in a stereotaxic frame. The body temperature was maintained between 35-37°C. Three small craniotomies and dural resections were designed for implantation of electrodes according to the atlas (Paxinos and Watson, 1982). A screw was fixed to the skull and used as a reference electrode. A glass microelectrode filled with 2 M NaCl for recording (DC resistance: 0.5-2 MΩ) was placed into the left frontal cortex (2 mm anterior and 3 mm lateral from bregma, and 0-0.5 mm deep from the cortical surface). A concentric electrode for thalamic stimulation and a bipolar electrode for callosal stimuli were inserted stereotaxically into the left ventrolateral nucleus (4 mm posterior and 2 mm lateral from bregma, and 4 mm deep from the cortical surface) and the callosal fibers in the right hemisphere (0-1 mm anterior and 1-2 mm lateral from bregma, and 2.0-2.5 mm deep from the cortical surface), respectively. Positions of electrodes were confirmed by monitoring thalamocortical (ThCRs) and transcallosal (TCRs) responses. During recording, the anesthetic level was kept at a level where a flapping response of the ear on pinching could be detected. For testing stimuli, 0.5 Hz rectangular pulses of 50 μs duration and 0.08-1.2 mA intensity were applied to the left ventrolateral nucleus, and ten successive ThCRs were digitized at 5kHz and averaged every minute throughout the experiment. For conditioning stimuli, 2.5, 5, 10 and 20 Hz rectangular pulses of

100 μs duration and 0.2-1.2 mA intensity were applied to the corpus callosum for ten minutes. An interval between conditioning and testing stimulation was 200 ms. The amplitude of the ThCR negative wave was measured and plotted before (preceding period), during (conditioning period) and after conditioning (recovery period). In order to observe the sequential changes of ThCR, the data were normalized with respect to the averaged values obtained during the ten-minute preceding period. For comparison, the data were also obtained from callosotomized rats with conditioning stimuli of 10 Hz. Callosotomy was performed paramedially and transcortically with a needle paying attention not to damage the cortical veins. Monitoring TCRs was used to determine the length and depth of sectioning. Recordings were initiated more than 30 minutes after each operation. Statistical analysis of the ThCR changes was carried out by the ANOVA and Turkey methods. The mean amplitude of the ThCR during conditioning period at different frequencies was also tested by the Mann-Whitney's *U*-test.

Results

Sequential changes throughout the experiment at a frequency of 10 Hz in normal rats are shown in Fig. 1A ($n = 12$). The amplitude of the ThCR during conditioning period was significantly larger than preceding and recovery periods ($P < 0.01$). ThCRs were immediately increased in amplitude when callosal stimuli were started, maintained at a constant level during conditioning period, and recovered to the original level upon cessation of the callosal stimuli. A similar facilitatory effect was also observed at 5 and 20 Hz ($n = 7$ and $n = 10$, respectively, $P < 0.05$), but no significant change was seen at 2.5 Hz ($n = 7$). Mean amplitudes of the ThCR during the conditioning period at different frequencies are shown in Fig. 2. There is an approximate positive correlation between the amplitude and the frequency from 2.5, 5 to 10 Hz. Values of the mean amplitude during 5 and 10 Hz conditioning were both significantly larger than that with 2.5 Hz conditioning ($P = 0.01$ and 0.001, respectively). However, a decrease in amplitude was observed when the frequency was further increased to 20 Hz. The mean amplitude during 20 Hz conditioning was significantly larger than that during 2.5 Hz ($P = 0.008$) but lower than 10 Hz ($P = 0.03$). On the other hand, no facilitatory effect of the transcallosal volley on the ThCR was observed in callosotomized rats ($n = 6$; at 10 Hz) (Fig. 1B).

Discussion

The present study shows that cortical reactivity, monitored by ThCRs, was enhanced by transcallosal volleys at frequencies of 5-20 Hz, and that this facilitatory effect was suppressed by callosotomy.

In contrast to the present findings, inhibitory actions of transcallosal volleys on the cerebral cortex were reported in the cat (Asanuma and Okuda, 1962) and human studies (Ferbert et al., 1992; Schnitzler et

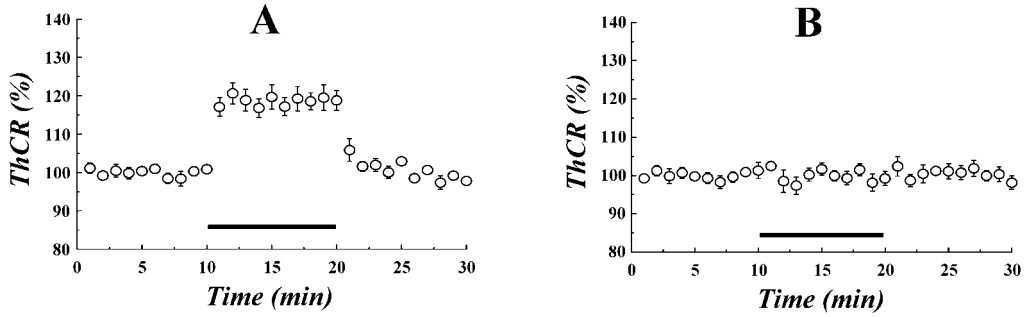

Fig. 1. Sequential changes of ThCR before, during and after callosal conditioning at frequency of 10 Hz in normal (A) (n = 12) and callosotomized rats (B) (n = 6).
Each point and bar represents the mean±SE. The thick horizontal bar indicates conditioning period. Amplitudes of ThCR were significantly higher during conditioning than pre-conditioning period in normal rats ($p < 0.01$, ANOVA-Turkey method). However, no significant change was observed in callosotomized ones.

al., 1996). Callosal fibers provide both excitatory and inhibitory connections to cortical neurons. The latter

was shown mainly to be mediated by surrounding interneurons (Asanuma and Okuda, 1962). On the other hand, repetitive and prolonged excitatory synaptic inputs reduced neuronal inhibitory actions (failure of inhibition) (Ben-Ari et al., 1979).

In hippocampal pyramidal cells, repetitive stimulation at 5-10 Hz depressed the inhibitory postsynaptic potentials and made the membrane potential less negative than the resting potential (McCarren and Alger, 1985; Thompson and Gähwiler, 1989). This observation supports our results, i.e. the facilitatory effect on the cortex due to continuous transcallosal volleys was anticipated at frequencies of 5-10 Hz. Thus, the failure of inhibition theory seems to account for our observations. The lesser degree of facilitation at 20 Hz may suggest depletion of neurotransmitters at callosal terminals due to high-frequency stimulation. Cortical neurons receive inputs mostly from the thalamus and other cortical areas, including the contralateral hemisphere. The ThCR was used as a measure of cortical reactivity for convenience in the present study, but cortical response to any inputs would be enhanced during bursts of transcallosal volley based on this hypothesized mechanism.

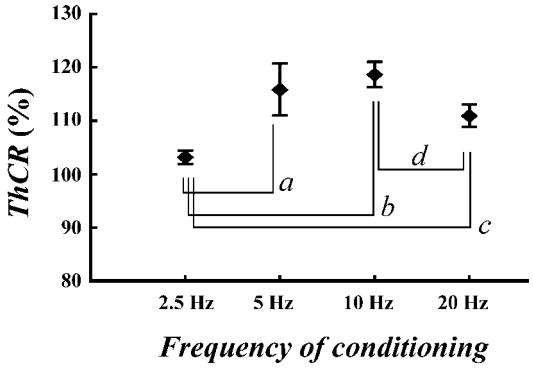

Fig. 2. Frequency analysis of transcallosal facilitatory effect on ThCR.
Mean amplitude of ThCR during conditioning period at frequency of 2.5 Hz (n = 7), 5 Hz (n = 7), 10 Hz (n = 12), and 20 Hz (n = 10) are compared in the graph (mean±SE). Significant differences between frequencies are shown. ($^{a}p = 0.05$, $^{b}p = 0.05$, $^{c}p = 0.05$, $^{d}p = 0.05$, Mann-Whitney's U-test).

In the epileptic brain, potentially predominant inhibition of the neuronal system, including commissural or callosal inhibitory action, was suggested (Maru and Goddard, 1987; Spencer, 1988). However, failure of inhibition was also regarded as a mechanism of seizure genesis and could release cortical neurons from suppression (Ben-Ari et al., 1979; Thompson and Gähwiler, 1989). Considering our findings, bursts of transcallosal volleys produced by contralateral cortical activity may make bilateral cerebral cortices more susceptible to seizure activity (enhancement of cortical reactivity) and drive generalized seizure discharges.

Bilateral symmetrical epileptic foci on the two hemispheres displayed the lowest threshold of potential epileptogenisity and more immediate mutual facilitatory influence between themselves (Rovit and Swiecicki, 1965) and resulted in generalized discharges (Murcus, 1985). A focus limited to one hemisphere could also produce similar ones (Murcus, 1985). In this situation, a massive transcallosal volley seems to underlie this. The present study supports these results and suggests a crucial role of the corpus callosum in interhemispheric facilitation, i.e., an induction and maintenance of seizure discharges. (McCaughran Jr., 1985).

Obviously, our experiments were carried out on the normal (i.e., non-epileptic) brain of the rat, and we have not directly measured cortical epileptogenesis. However, if the cortical reactivity to the thalamic input shares a common neuronal mechanism, at least in part, with that of the epileptic discharge generation (Kostopoulos and Avoli, 1983), it seems probable that the bilateral cerebral cortices facilitate each other through transcallosal connections and that the callosotomy can suppress this interaction, causing a reduction of the cortical excitability itself. This can also reasonably account for most of the surgical effects after callosotomy, i.e., reducing seizure frequency, severity, and EEG discharges.

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