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Effects of Pilocarpine and Kainic Acid on EEG and Behavior Activity in Freely Behaving Rats

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Abstract – This study was undertaken to evaluate a behavior-electroencephalogram (EEG) pattern relationship in pilocarpine- and kainic acid-induced convulsions of rats. Also we intended to examine the effect of a noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist, MK-801, and diazepam on the pilocarpine-induced behavioral and electrical seizures in rats. The electrical activities at frontal and hippocampal areas and behavior activities were measured in freely moving rats. At the beginning of the experiments, the rats displayed an exploratory behavior. This awake and moving phase with a low amplitude, irregular, 4-10 Hz wave was followed by a still phase. Pilocarpine (400 mg/kg, i.p.) and kainic acid (0.5 mg/kg, i.c.v.) induced tonic and clonic seizures. The pilocarpine-induced change in electrical activities exhibited a weak correlation with behavioral convulsion at all stages. The amplitude and duration of the electrical response were not linear with the degree of behavioral score. An application of MK-801 (dizocilpine, 0.5 mg/kg) did not affect the amplitudes of the convulsant-induced electrical activities, though the same dose of this drug caused the deformation of the electrical pattern. There was no effect of MK-801 on the behavioral and electrical activities as expected. Diazepam (1 mg/kg) did not affect the amplitude of the electrical activities induced by pilocarpine but changed the pattern of these activities. Our study shows that there is no linear relationship between degree of behavior and amplitude of electrical activities of convulsants. This may indicate that the NMDA receptor stimulation can be processed by the neocortical or hippocampal network in a different way between behavioral and electrical activities.

Keywords □ pilocarpine, kainic acid, MK-801, diazepam, EEG, ECoG, behavior.

Pilocarpine has been regarded as a principal convulsant in the central nervous system. Several kinds of responses induced by pilocarpine have also been related with GABA- (Turski *et al.*, 1991; Patel *et al.*, 1987), dopamine- (Barone *et al.*, 1991) and glutamate-containing neurons (Turski *et al.*, 1987). Pilocarpine-induced brain damage was produced by operating NMDA receptor-activated channels which permit the influx of calcium as well as sodium. The overstimulation of this type of receptor is one mechanism for calcium overload in neurons (MacDermott *et al.*, 1986). NMDA receptors play a key role in the mediation of excitatory synaptic signals in the brain (Collingridge and lester, 1989; Dale, 1989; Stone and Burton, 1988). It has conclusively shown with extra- and intracellular recording techniques in several *in vitro* preparations that excitatory postsynaptic potentials (EPSP) in most of the neurons contain a component with characteristics of an NMDA receptor-mediated event (Dingledine, 1983; Herron *et al.*, 1986; Pierson *et al.*, 1989; Thomson, 1986). However, the NMDA-sensitive EPSP may contribute to action potential generations in both inhibitory and excitatory neurons, and such firings may activate various feed-back mechanisms. Thus we can pose a question that NMDA receptor stimulation at the synaptic level necessarily promote excitation at the system level in intact neural network. According to *in vivo* studies in the cases of many brain structures, NMDA receptor-mediated synaptic and cellular events can lead to excessive excitation in the local networks of several brain areas.

Interestingly, in neocortex, the relationship between NMDA receptor-mediated events at the synaptic level and those at the network level is not as straightforward as in the cases of hippocampus or brainstem structures.

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Single cell studies on neocortical slice preparation have demonstrated that these receptors are involved in EPSP generation (Thomson, 1986) and induction of repetitive neuronal firing (Flatman *et al.*, 1983). EEG analysis of the effects of NMDA in the cerebral cortex of freely behaving animals is not easy. Because of this, there is lack of information about the electroencephalographic correlates of NMDA-related behavioral events taking place at *in vivo* level. In fact, it is not known whether in cortex, *in vivo*, epileptiform electrical activity appears in any phase of the NMDA action. Therefore, the role of NMDA receptors in neocortical epileptogenesis is much less understood than their role in seizures emanating from subcortical structures (Coutinho-Netto *et al.*, 1981; Croucher *et al.*, 1982; McNamara *et al.*, 1988). The present study addressed basic issues pertinent to NMDA receptor-mediated action in the neocortex and hippocampus, by using the combined EEG-behavioral analysis technique in freely moving rats. This method was used because it offered the unique advantage of recording the local EEG, with the simultaneous monitoring of the behavioral activity. Our principal goals were to determine the behavioral and electrographic effects of convulsants in the absence and presence of the non-competitive NMDA receptor antagonist MK-801 and benzodiazepine receptor agonist diazepam.

MATERIALS AND METHODS

Animals and surgery

Male Sprague-Dawley rats (200-280 g) were used. After the operation, they were housed individually and allowed *ad libitum* access to food. Some of these animals were used for methodological tests to determine the dose and injection time of convulsants and the optimal parameters for this study. Each rat was subjected to one experimental session only. The rats were anesthetized with a mixture of ketamine (150 mg/kg, i.p.) and tridol (25 mg/kg, i.p.), and operated in a stereotaxic apparartus. Animals that drugs would be given via i.c.v. route were implanted with a guide cannula (22-gauge stainless steel tube) placed near the ventricle (P 0.8 mm from bregma; L 1.5 mm from midline; V 3.0 mm from dura mater). A stlylet was inserted into the cannula and remained there at all times except during i.c.v. injection. Four screws were placed on the skull, and the recording electrode was streotaxically placed in the neocortex or hippocampus, if necessary. Coordinates for neocortex were : A 2.0 mm (from bregma), P 3.8 mm (from midline), L 2. 0 mm and V 0.5 mm (from dura mater), and those for hippocampus were : P 3.8 mm (from bregma); L 2.0 mm (from midline) and V 3.5 mm (from dura mater), according to Paxinos and Watson (1986). The ground wire was connected to one of the screws, and the whole headstage assembly was anchored to the skull with dental acrylic cement (Fig. 1). The rats were introduced into the experimental sessions after a 5-day postsurgical recovery.

Electrophysiological data acquisition and analysis

The EEG from the four fixed electrodes in the skull was recorded monopolarly with respect to the indifferent screw electrode by an Axon polygraph (1.0-200 Hz). The EEG recording session was carried out before and immediately after drug administration until the rat showed convulsion. The connector of the recording cable was plugged into the headstage connector and electrophysiological data acquisition was started. The raw EEG waves were continuously displayed on a computer monitor. The electrophysiological data were stored on a hard disk. Artifact-free recordings were made with the use of a preamplifier built in the recording cable.

Behavioral monitoring

Through the 5-hr experimental sessions after injection of drugs, the rats were moving freely in the test chamber and observed for signs of seizure activity. Seizure severity was scored on the following rating scale: 0=no convulsion; 1=tremor, head bobbing, backward walking, wet dog shakes; 2=intermittent forepaw myoclonus, rearing and falling; 3=continuous clonic convulsion; 4=tonic flexion; 5=respiratory arrest.

Drugs

Pilocarpine hydrochloride and kainic acid were obtained from Sigma chemical Co., and diazepam and (+)-MK-801 hydrogen maleate were from RBI and disssolved in saline.

RESULTS

Behavioral and electrical activity relationship for kainic acid response

At the beginning of the experiments, the rats displayed an exploratory behavior. This awake and moving phase with a low amplitude, irregular, and 4~10 Hz wave was followed by a 20~30 min awake but still phase, after which most of the rats fell asleep. Administration of kain-

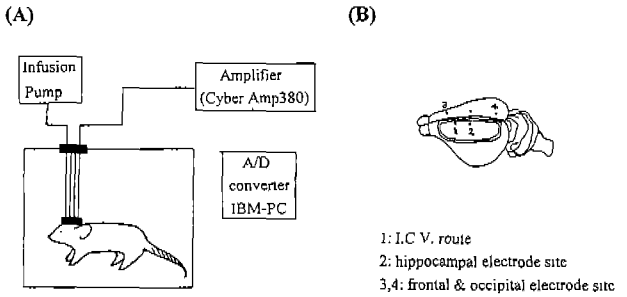

Fig. 1. Schematic diagram of experimental apparatus used in this study (A) and recording points on the surface of the rat brain (B). Note that a preamplifier was utilized for eliminating movement artifacts from EEG recordings.

ic acid resulted in the development of a characteristic excitotoxic syndrome, consisting of automatisms and wet dog shakes, which then progressed to convulsive activity with waxing and waning behavioral and electrographic severity. Finally, a continuous convulsive state ensued, combined electrographically with continuous fast spiking. This stage was followed by a dramatic electrographic change to periodic epileptiform discharges. Fig. 2 shows changes in the electrocorticogram (ECoG) and hippocampal EEG induced by kainic acid at various behavioral degrees between S0 and S4. The kainic acid-induced changes in electrical activities exhibited a weak correlation with behavioral convulsion at all stages. The amplitude and duration of the electrical response were not linear with the degree of behavioral score. These findings mean that the severity of behavioral activities does not always coincide with the degree of electrical activities.

Effect of NMDA receptor antagonist

Since pilocarpine increases the release of excitatory amino acid neurotrasmitters such as glutamate, seizures induced by this agent is thought to be due, at least in part, to excessive activation of glutamate receptors, particularly of the NMDA subtype. Thus we examined the effect of MK-801, uncompetitive NMDA antagonist, in the pilocarpine model of seizures in rats. The relationship between the degree of the behavioral activity and amplitude of electrical convulsion is shown in Fig. 3, in which MK-801 pretreatment was conducted 15 minutes before convulsant administration. Convulsant itself did not increase the amplitude of electrical activities in a behavioral activity-dependent manner in the seizure range between S0 and S1. At behavioral degrees of S3, S4 and S5, pilocarpine induced a weak electrical activities. Each behavioral seizure response had not a characteristic EEG correlate. The seizure occurred 1-3 min after the drug injection. Contrary to expectation, MK-801 failed to protect rat against electrical and motor seizure induced by pilocarpine. Applications of MK-801 did not affect the amplitudes of the convulsant-induced electrical activities, though the same dose of this drug caused the deformation of the electrical pattern. The degrees of muscle spasms and tonic-clonic seizures induced by pilocarpine are not reduced after applicaltion of an MK-801.

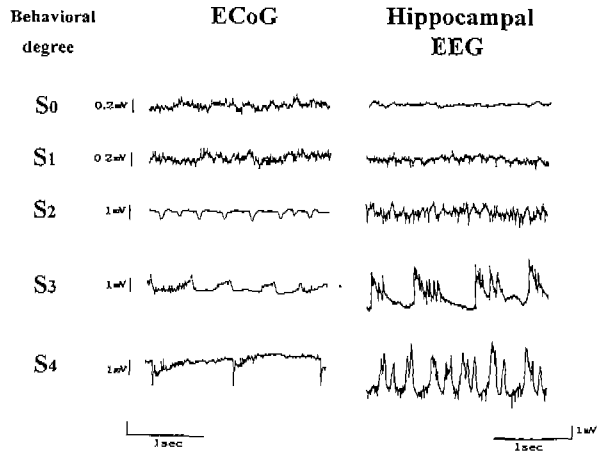

Fig. 2. The EEG recordings from hippocampal and frontal area in kainic acid (0.5 mg/kg, i.c.v.)-induced convulsion of rat. EEG patterns were matched with behavioral seizure. Behavioral degree was defined as following: S0 no convulsion; S1 tremor, head bobbing; S2 hind leg myoclonus; S3 continuous clonic convulsion; S4 tonic convulsion; S5 respiratory arrest.

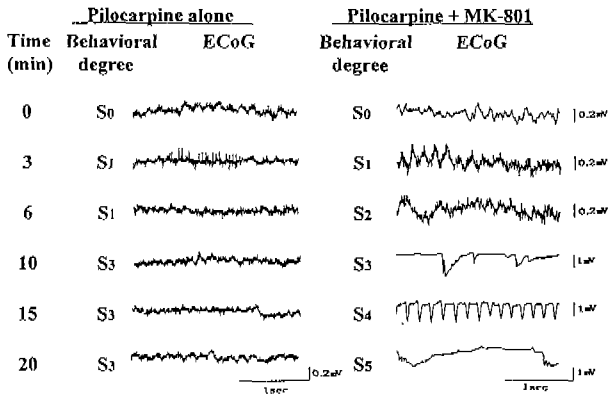

Fig. 3. The EEG patterns from frontal area in pilocarpine (400 mg/kg, i.p.)-induced convulsion after pretreatment with MK-801 (0.5 mg/kg, i.p.) in rat. MK-801 pretreatment was conducted 15 minutes before pilocarpine administration.

Effect of benzodiazepine receptor agonist

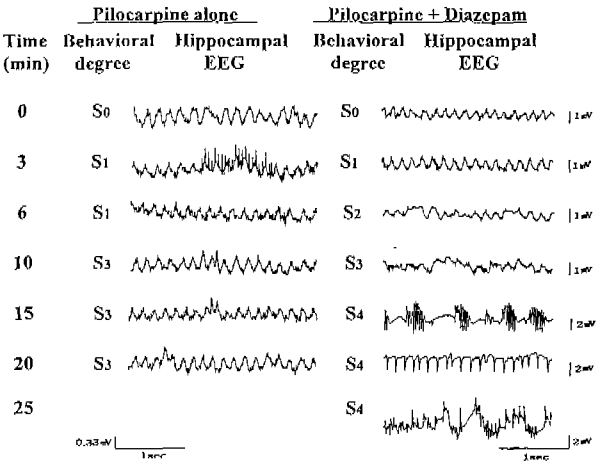

Fig. 4. The EEG patterns from hippocampus in pilocarpine (400 mg/kg, i.p.)-induced convulsion after pretreatment with diazepam (1 mg/kg, i.p.) in rat. Diazepam pretreatment was conducted 15 minutes before pilocarpine administration.

Because the sign and the symptom of convulsion are closely related with the state of GABAergic synaptic inhibition, it will be necessary to investigate the role of benzodiazepines which increase the efficiency of GABAergic action in seizure activities. Therefore, we examined the effect of diazepam, a benzodiazepine receptor agonist, on the pilocarpine-induced convulsion to confirm whether electrical and/or behavioral seizure could be protected by this agent. Fig. 4 shows the effect of diazepam on the relationship between behavioral and electrical activity induced by pilocarpine. At this experimental condition, diazepam did not suppressed the neocortical discharges, such as continuous spiking or ictal activity, induced by pilocarpine but changed the pattern of these activities at which fast spiking became interrupted by flat periods and periodic complexes soon dominated the electroencephalogram in bilateral neocortical foci. Behaviorally, rhythmic tonic- and clonic-hyperactivation still prevailed by sequential administration of diazepam and pilocarpine, but to a lesser degree than by pilocarpine alone, which increased the number of episode.

DISCUSSION

Anatomy of seizure activity in stages of seizures involves many structures. Specially, orbital, prefrontal and insular cortical areas are activated and numerous limbic areas and hippocampus are partly activated. These activated cortical and limbic regions are known to possess extensive interconnections (Krushel and van der Kooy, 1988), hence their conjoint activation is consistent with seizure propagation along anatomic pathways. In our results, the activations of hippocampl EEG were similar with those of ECoG. These activation sequences are compatible with hippocampal formation being activated simultaneously with frontal cortex involvement. This results mean that cortical involvement spread to bilateral areas so that the entire cortical mantle become involved. Correspondingly, anatomically linked thalamic and hippocampal structures are activated.

Clifford *et al.* (1987) reported that despite almost total involvement of the cerebral hemispheres by seizure activity, animals were capable of locomotion and react to tactile and acoustic stimuli. These findings support our results that changes in electrical activities exhibit a weak correlation with behavioral convulsion at all stages. An explanation of this difference between generalized electrical seizure and behaviors may reside in the difference in electrographic spiking rates. Therefore, the barrage of cortically propagated epileptiform discharges may be occurring at such a rapid rate in our experimental model that the motor pattern generators in brainstem and spinal cord fail to respond. In support of this postulate is the report that slower electrographic spiking in lithium-pilocaprine status epilepticus stages is correlated with increased convulsive severity, as assessed by the amplitude of convlusive movements and the loss of ambulation and of reflex responses to sensory stimuli (Handforth and Treiman, 1995). Presumably, this slower activity is now capable of engaging pattern generators so that behavior is dominated by convulsions.

The sensitivity of pilocarpine-induced seizures to NMDA receptor blockade with MK-801 which had anticonvulsive properties was studied in freely behaving rats. Contrary to expectation, MK-801 failed to protect rats against motor seizure induced by pilocaprine. It is known that pilocarpin increases electrical and behavioral activities by cholinergic activation and involves NMDA receptor stimulation. If so, the seizures induced by NMDA receptor activation will be counteracted by pretreatment of MK-801. Although the mechanism underlying these paradoxical results of our experiments is unknown, our results are consistent with the findings that applications of glutamate or NMDA on the cortical surface do not cause epileptic events (Rogers *at al.*, 1989; Van Harreveld, 1959) and that NMDA receptors can be

involved in inhibitory processes, at network level, in the intact neocortex (Addae and Stone, 1986; Kent and Webster, 1986). Also, our data are in agreement with the observation of Alberici *et al.* (1989) that systemic administration of subconvulsive doses of NMDA in rats decreases the amplitude of the electrocorticogram and shifts the power spectrum towards the delta band. These EEG changes apparently reflect depressant cortical NMDA actions not overwhelmed by the subcortical excitatiory effects of the drug. Furthermore, our study supports the hypothesis of Marrannes *et al.* (1988) that NMDA receptors may be involved in the pathophysiology of cortical spreading depression, because many characteristics of NMDA stimulation action, such as EEG amplitude discordance with behavioral activity, resembled those that occured during spreading depression (Leão, 1972). Since, at the synaptic level, NMDA receptors mediate excitatory signals in neocortex (Flatman *et al.*, 1983; Thomson, 1986), the paradoxical EEG depressant effect of the stimulation of these receptors at the network level is probably related to a unique cascade of events that follows the initial cellular excitation. As pointed out by Krnjevic (1970) and Munoz-Martinez (1970), the excitatory effects of glutamate can be so rapid and strong that it can cause prolonged depolarizations followed by neuronal exhaustion and concomitant termination of action potential generation. Undoubtedly, similar cellular events may underlie the NMDA-induced cortrical EEG depression. Ludvig *et al.* (1992) showed that in the hippocampus, NMDA could trigger and maintain epileptiform spike activity. This indicates that massive neuronal depolarizations mediated by NMDA receptors do not necessarily induce secondary functional block in a neuronal circuitry. The present data suggest proconvulsant properties of MK-801 and limitations of its clinical utility as an antiepilectic agent.

We examined the effect of diazepam, a benzodiazepine receptor agonist, on the pilocarpine-induced seizure to confirm the effect of indirect glutamate receptor stimulation on GABAergic inhibitory interneuron. The principal finding of this study was that in the freely behaving rats, diazepam did not suppressed the neocortical discharges induced by pilocapine. The possibility that these results come from neural network connection can not be excluded because it is possible that in neocortex, GABA receptors are preferentially localized on inhibitory interneurons. Activation of these receptors would result in a disinhibition in the circuitry, via an decreased firing of inhibitory neurons. Although glutamatergic/aspartatergic cortico-cortical neurons (Giuffrida and Rustioni, 1989) may form NMDA receptor-utilizing synapses on inhibitory interneurons in a high quantity, no information is available on the distribution of NMDA receptors on the somata and dendrites of varous types of cortical neurons.

Alternatively, a special GABA receptor population may be present on the axon terminals containing inhibitory neurotransmitters. In this case, GABA receptor stimulation might result in an decreased outflow of inhibitory substances, which also could not lead to a widespread inhibition. Although NMDA has been reported to facilitate the release of GABA in neocortical cell cultures (Drejer *et al.*, 1987), the existence of presynaptic NMDA receptors is uncertain (Fagg and Matus, 1984; Stone and Burton, 1988).

It is also possible that stimulation of GABA receptors in cortical neurons activate powerful negative feedback mechanisms, at either the network or synaptic level, or at both. At the synaptic level, perturbations of the Cl^- or cyclic nucleotide second messenger systems following GABA channel-gating (zorumsky and Isenberg, 1991) may trigger some special regulatory processes that could suppress the primary IPSPs. Further studies, including *in vivo* single cell recordings and GABA release measurements during cortical NMDA action, are required to pin-point which of the above or other unknown processes operate in nature.

The effects of pilocarpine and kainate in the cortex and hippocampus indicate that the physiological roles of NMDA and GABA receptors in signs and symptoms of convulsion induced by these agents are probably different. Our experiments praticularly suggest a divergent role for the NMDA receptor machinery in seizures of cortical and hippocampal area. In neocortex, NMDA receptor inactivation apparently can not force the spike-generator neuron population to fire in epileptiform modes. This shows a similarity with the situation in the hippocampus, but sharp contrast with brainstem nuclei, where the spike-generator neurons can be readily overexcited and rendered epileptic events by NMDA receptor stimulations (Millan *et al.*, 1986; Rogers *et al.*, 1989). However, neocortical NMDA and GABA receptors may have important modulatory functions in the propagation and termination of various types of seizures in brain, like

the striatal NMDA receptors of which stimulation protects against limbic seizures (Turski *et al.*, 1987). Thus, there is another possibility that the neocortical neurons in which NMDA receptor is activated have low sensitivity to input from the hippocampal neurons. To confirm this possibility, further experiments will be needed.

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