Amino Acids (1992) 3: 119–124

Amino
Acids

Bicuculline-induced blockade of neocortical rapid kindling suggesting facilitative GABAergic action on seizure development

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Accepted October 10, 1991

Summary. To investigate how GABAergic function affects seizure development, the effects of a GABA antagonist, bicuculline, on neocortical and hippocampal kindling were examined in chronically prepared rabbits. Kindling-inducing stimulations consisted of stimulus trains repeated at 5-min interstimulus intervals to produce so-called "rapid kindling". The changes in after-discharge (AD) durations induced by each of 15 trials of stimulus trains per session were compared before and 30 min after i.p. injection of bicuculline solution (2 mg/kg) in each of three kindling groups consisting of 5 rabbits each, i.e. visual cortical, motor cortical and hippocampal kindling groups. In the visual cortex and to a less extent, the motor cortex kindling groups, the AD durations were shortened after bicuculline injection and did not show the progressive prolongation seen before the injection. In contrast, the hippocampal kindling group showed a further marked prolongation of the AD durations after the injection. The bicuculline-induced blockade of neocortical kindling suggests facilitative GABAergic action on seizure development, while the drug-induced enhancement of hippocampal kindling reflects the known inhibitory GABAergic action.

Keywords: Amino acids – Neocortex – GABA – Kindling – Rabbits

Introduction

It has long been controversial in the field of epilepsy which abnormality of excitatory and inhibitory neuronal functions is responsible for seizure development. Concerning the former excitatory function, we have recently found in a study of kindling as a model of seizure development that activation of N-methyl-D-aspartate (NMDA) receptors contributes substantially to the kindling-induced potentiation of excitatory synaptic transmission (Jibiki et al., 1990 and 1991a). In the present study, concerning the latter inhibitory function, we investigated how GABA-ergic function, known to be a major inhibitor of synaptic

function, affects seizure development, by observations of the effects induced by a GABA antagonist, bicuculline, on neocortical and hippocampal kindling.

Material and methods

Chronic experiments were performed on 20 adult male rabbits each weighing 2.5–3.0 kg. These rabbits were divided into four groups of 5 rabbits each, i.e. visual cortical (VC), motor cortical (MC) and hippocampal (H) kindling groups, and a control group. Operation was conducted under i.v. pentobarbital sodium anesthesia (20 mg/kg). According to Rose's map (Rose and Rose, 1933), a concentric bipolar stimulating electrode was inserted into the superficial layers of the right visual and motor cortices in each of the VC and MC groups, respectively, viewing these exposed cortices in the face after craniectomy and incision of the dura. In the H group, a pair of stimulating and recording electrodes was inserted from the pial surface at the position of P4 and L6 on Ridge's map (Ridge, 1964) to the right dorsal hippocampus (CA1) using an oil hydraulic microdrive (Narishige), with laminal analysis every 50 or 100 μm (Jibiki et al., 1990 and 1991a). In all of the 5 rabbits of the H group, the depths of the inserted hippocampal sites measured 2600–3500 (3050 $\pm$ 315) μm below the surface of the cerebral cortex. In the control group, the stimulating electrode was inserted into the right visual cortex as in the VC group. These electrodes were fixed to the skull with dental cement. For EEG recording, stainless-steel screw electrodes were placed symmetrically on the bilateral motor, parietal and visual regions of the skull. Both the EEG recording and hippocampal depth recording were referred to a screw electrode placed on the frontal sinus.

Experiments were conducted after a postsurgical recovery period of 2 weeks. Initially, the threshold intensity to induce after-discharges (ADs) localized in each stimulated site was examined by changing the stimulus currents alone. Then, stimulus trains at a constant intensity, which were decided as 100 μA above the threshold current, were repeatedly applied at 5-min intervals. The parameters consisted of monopolar square pulses of 1 msec duration, 200–1000 μA, 60 Hz and 1 sec in total duration in all of the rabbits. The stimulus trains were repeated 15 times to induce so-called "rapid kindling", which signifies progressive seizure development produced by electrical stimulations repeated at short interstimulus intervals in chronic preparations (Lothman et al., 1985; Jibiki et al., 1991b). Soon after rapid kindling was observed, bicuculline 2 mg/kg dissolved in Ringer's solution 5 ml and propylene glycol 1 ml was injected intraperitoneally. Thirty minutes after the injection, stimulus trains of the same intensity as used previously were again repeated 15 times. Changes in rapid kindling, especially changes in the development of AD duration induced by each stimulation, were compared before and after the bicuculline injection in each of the VC, MC and H groups. In the control group, however, the vehicle alone without bicuculline was injected.

After the termination of the experiments, each stimulated site was identified histologically by examining the traces of the electrode tip.

Results

In the VC, MC and H groups, the threshold stimulus currents to induce ADs localized in each stimulated site, were 0.5 $\pm$ 0.3, 0.8 $\pm$ 0.1 and 0.4 $\pm$ 0.3 μA, respectively, showing that the threhold intensities were significantly lower in the visual cortex and hippocampus than in the motor cortex (two-tailed t-test, $p < 0.05$). Furthermore, the AD durations elicited by the first trial of the kindling-inducing stimulations at suprathreshold intensities were 7.2 $\pm$ 3.1, 11.2 $\pm$ 3.1 and 13.8 $\pm$ 6.7 sec, respectively, in the VC, MC and H groups.

The 15 trials of the kindling-inducing stimulations at 5-min intervals prior to bicuculline injection produced progressive prolongation of AD durations,

interposed, however, by occasional misfiring or shortening of ADs, and gradual development of propagated seizure discharges in cortical areas other than the stimulated site. Such kindling development was relatively clear in the VC group, while the H group often showed undeveloped kindling in which brief ADs of almost identical durations were continuously elicited (Fig. 1A and C, before bicuculline injection). The MC group usually exhibited kindling development intermediate between that seen in the VC and H groups (Fig. 1B, before bicuculline injection).

The 15 trials of kindling-inducing stimulations after bicuculline injection revealed a suppression of kindling development in the VC and MC groups: the AD durations elicited by each stimulus were shortened and did not show the

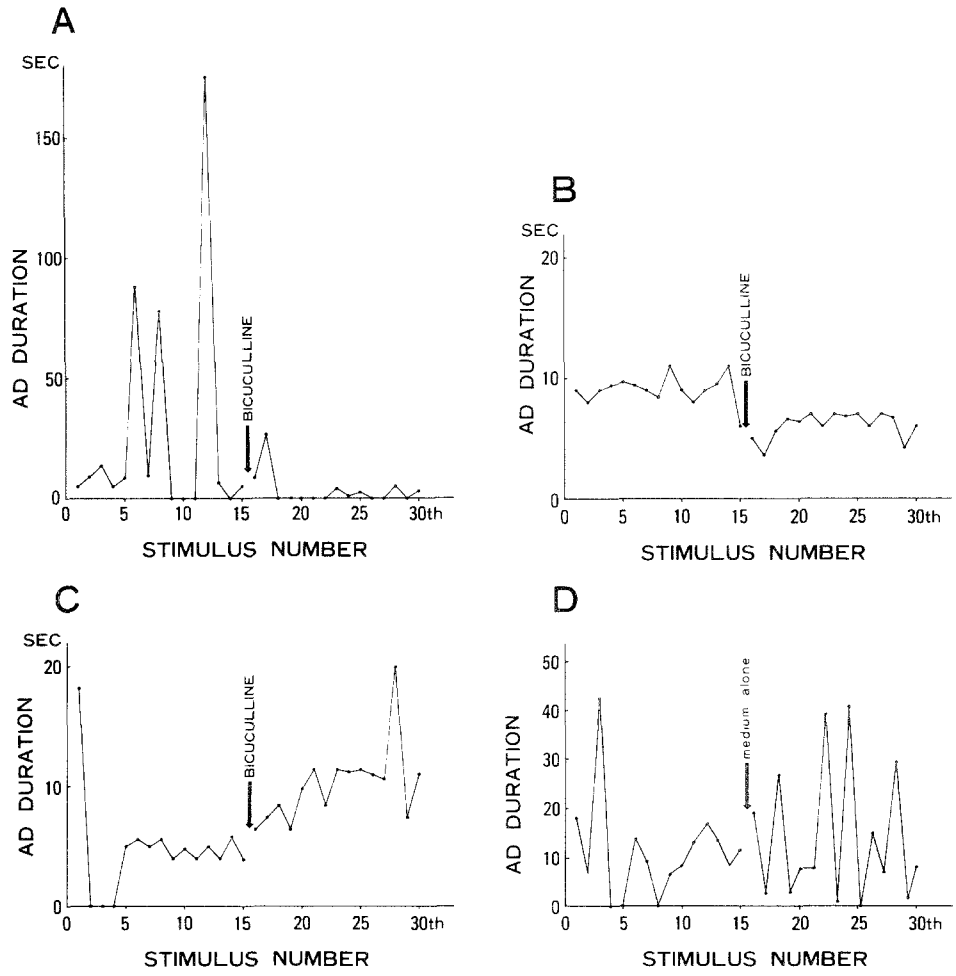

Fig. 1. Changes of AD durations in kindling produced by 15 stimulus trials repeated at 5-min intervals before and after bicuculline i.p. injection. **A**, **B** and **C** show a typical example from the VC, MC and H groups, respectively, in which the right visual and motor cortices, and the right hippocampus (CA1) were kindled, respectively. **D** shows an example from the control group, in which the right visual cortex was kindled and vehicle alone without bicuculline was injected. Stimulus trials after bicuculline or medium injection were started 30 min after the injection

progressive prolongation which was seen before the injection (Fig. 1A and B, after bicuculline injection). Furthermore, the concomitant development of propagated seizure discharges was diminished. Such supression was more marked in the VC group than in the MC group. In contrast, the hippocampal kindling group showed more marked prolongation of the AD durations after the injection than before the injection (Fig. 1C, after bicuculine injection). The concomitant development of propagated seizure discharges, too, was enhanced still more after as compared with before the injection.

On the other hand, the control group, in which the visual cortex was kindled as in the VC group, showed an almost equivalent or slightly more advanced kindling development, consisting of mainly AD development, after vehicle injection, as compared with before the injection (Fig. 1D).

With regard to each of the VC, MC, H and control groups composed of 5 rabbits each, the mean and standard error of AD durations induced by 15 stimulus trials were calculated separately before and after bicuculline injection. The VC and H groups alone showed significant differences between these values

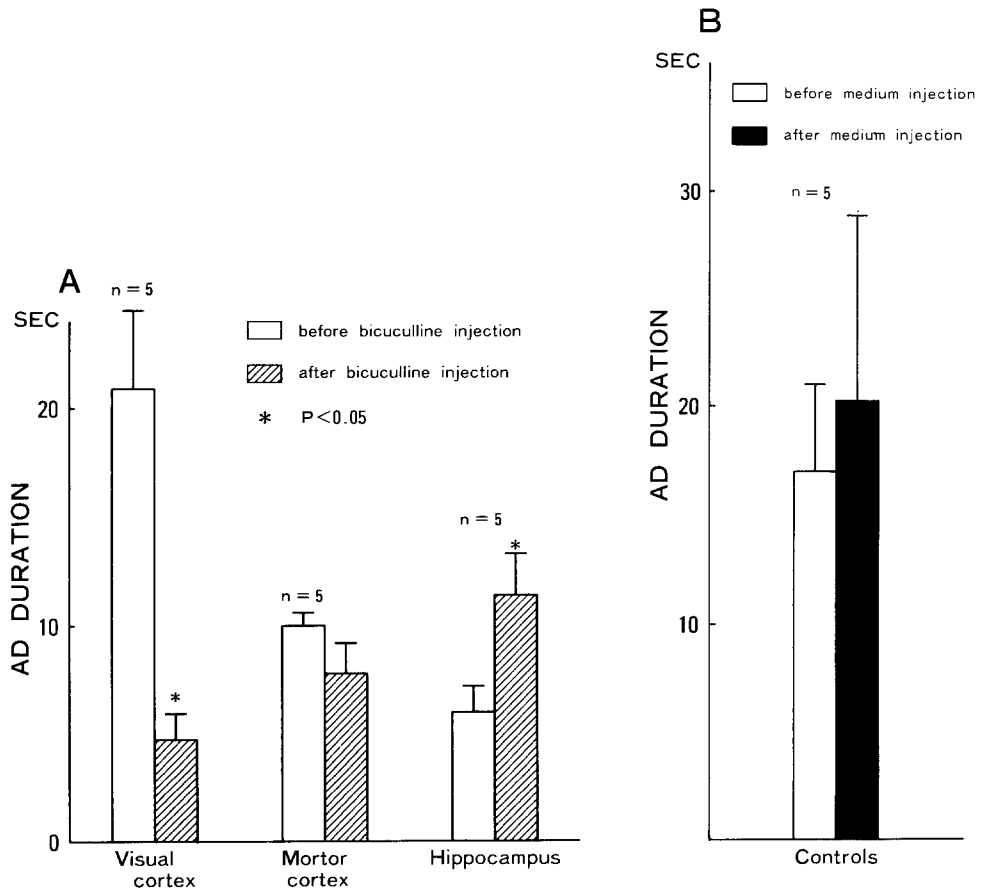

Fig. 2. A The mean and standard error of AD durations induced by 15 stimulus trials before and after bicuculline injection in the VC, MC and H groups composed of 5 rabbits each. **B** The mean and standard error of AD durations induced by 15 stimulus trials before and after vehicle injection in the control group. Asterisk denotes a significant difference between these values before and after the injection (two-tailed t-test, $P < 0.05$)

before and after the injection (two-tailed t-test, $P < 0.05$), showing, respectively, a significant decrease and increase of AD durations after the injection (Fig. 2).

Discussion

The present study showed that a GABA antagonist, bicuculline, had inhibitory effects on visual and motor cortical kindling, with facilitative effects on hippocampal kindling. To our knowledge, no reports have described such bicuculline-induced effects on kindling. It is widely recognized that GABA or GABA agonists inhibit kindling and various types of drug-induced seizures, and also, that GABA antagonists such as bicuculline, picrotoxin and penicilline induce seizures (Delgado-Escueta et al., 1986). Accordingly, the present facilitative effects of bicuculline on hippocampal kindling were anticipated, while the inhibitory effects of this agent on neocortical kindling were unexpected.

The present bicuculline-induced inhibitory effect on neocortical kindling suggests a facilitative GABAergic action on seizure development. As to the underlying mechanisms of such effect, the following three possibilities are considered. First, it is known that, in the local circuit in the neocortex and hippocampus, one GABAergic interneuron such as the Basket cell or stellate cell receives inputs from another GABAergic interneuron, whose inhibitory system delicately regulates the excitability of the pyramidal cells (Roberts, 1986). Bicuculline may block such an inhibition of inhibition, leading to enhanced inhibition of the pyramidal cells. Second, it is known from in vitro studies in the hippocampus that GABAergic depolarization arises in the dendrite of the pyramidal cell, whereas GABAergic hyperpolarization usually occurs in the somata of the pyramidal cell (Andersen et al., 1979). The neocortex also has abundant GABAergic synapses not only in layer IV, i.e. the somata layer but also in layer I, i.e. the dendrite layer (Sivilotti and Nistri, 1991). Therefore, the GABAergic depolarization may arise also in the neocortex. Bicuculline may block such GABAergic depolarization, and resultantly decrease the excitability of the pyramidal cells. Third, it has been recently found from in vitro or in vivo studies in the neocortex and hippocampus that GABA augments excitatory glutamate responses by allosteric modulation on glutamate receptors, which results from the structural similarity of glutamate and GABA receptors (Walden et al., 1989, 1990). Bicuculline may also act on the glutamate receptors, eventually leading to suppression of the excitatory responses.

With respect to these three possibilities, no presently available evidence can explain the present regional differences in bicuculline-induced effects between neocortical and hippocampal kindling. In the present study, AD thresholds were lower in the visual cortex and hippocampus than in the motor cortex. This finding is consistent with the results of our previous study (Kubota, et al., 1987). One may argue that bicuculline-induced facilitation of hippocampal kindling is due to the lower AD thresholds, i.e. higher seizure susceptibility in the hippocampus. However, although the AD thresholds in the visual cortex showed no significant difference as compared with those in the hippocampus, the visual cortical kindling was not facilitated but suppressed by bicuculline. Furthermore, although the AD thresholds in the visual cortex were significantly lower than

those in the motor cortex, visual cortical kindling was more markedly suppressed by bicuculline than motor cortical kindling. Therefore, the regional difference in AD thresholds does not explain the present regional difference in bicuculline-induced effects. A satisfactory explanation for the present regional difference in bicuculline-induced effects must thus await more detailed studies, although the present findings may shed new light on GABAergic action on epileptic activity.

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Received July 17, 1991