# CONCENTRATION- AND TIME-DEPENDENT EFFECT OF AMINOOXYACETIC ACID ON CORTICAL EPILEPTOGENICITY

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In the present electrophysiological study the effect of aminooxyacetic acid (AOAA) on the cortical epileptogenicity, and on the basic electro-cortical activity was investigated in anesthetized rats.

AOAA did not induce spontaneous epileptiform discharges but modified the somato-sensory evoked responses and the cortical epileptogenicity (induced by 4-aminopyridine) in the same manner depending on its concentration. AOAA at low concentrations increased the amplitude of evoked responses and the ipsilateral manifestation of epileptiform activity, however, at high concentrations significantly suppressed both the evoked responses and the induction and expression of seizures discharges. The anti-convulsive effect of AOAA was time-dependent (reached its maximum after 2h AOAA pre-treatment) and reversible. AOAA at low concentrations probably increases the efficacy of the NMDA excitatory system and decreases GABA-synthesis, resulting neuronal hyperexcitation. However, AOAA at high concentrations can lead to an effective cortical inhibition through intra- and extracellular accumulation of GABA. The gradual GABA accumulation – up to a certain level – at the synapses could also explain the time-dependency of the anticonvulsive effect of AOAA.

*Keywords:* Aminooxyacetic acid – anticonvulsive – proconvulsive – 4-AP-induced seizures

## INTRODUCTION

Aminooxyacetic acid (AOAA) is a non-selective inhibitor of several pyridoxal phosphate-dependent enzymes in the brain [27, 28, 29]. The various effects of AOAA at different experimental conditions, include: (i) blocking of GABA catabolizing enzyme (GABA-transaminase, GABA-T) [3, 15, 16], GABA-synthesizing enzyme (glutamate-decarboxylase, GAD) [31, 32], as well as kynurenine-transaminase [7, 18, 23, 29]; (ii) influencing of GABA release and uptake [3, 19, 22]; or (iii) confusing the intracellular energy metabolism [30] make AOAA possible to modify the physiological balance of excitation and inhibition in the nervous system.

Multiple and controversial effects of AOAA on epileptogenesis were reported in several studies which can be explained by the fact that AOAA through its complex biochemical effect can influence both excitatory and inhibitory neurotransmissions.

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In penicillin-, kindling- or genetic epilepsy models, AOAA was found to be anti-convulsive by suppressing the ongoing seizure activity or preventing the induction of seizure discharges [3, 8, 11, 13, 14, 15], however, in other experimental conditions AOAA proved to be proconvulsive by inducing and/or facilitating epileptiform discharges, behavioral seizures [4, 6, 17, 27, 28].

Earlier experiments on the effect of AOAA on epileptogenicity, were carried out on different species and different epilepsy models so far, with variable administration techniques and concentrations of AOAA. Therefore, in the present study our aim was to compare the different concentration- and time-dependent effects of AOAA on the same experimental epilepsy model (induced by 4-aminopyridine (4-AP)), and on the physiological functions of the cortex, like the basic electrocortical activity and somato-sensory evoked responses.

## MATERIALS AND METHODS

Electrophysiological experiments were carried out on adult (body weight: 240–280 g) Wistar rats (n = 31) of either sex. Under general anesthesia (sodium pentobarbital 50 mg/kg, i.p.) the dura was removed on the somato-sensory cortex of the right hemisphere, and the epileptiform activity was induced by local application of 4-AP. 4-AP crystal was applied to the exposed cortical surface on a filter paper soaked with saline. The electrocorticographic (ECoG) activity was recorded by 4 ball-tipped silver wire electrodes from the site of 4-AP application (primary focus, Pf), from the contralateral homotopic point (mirror focus, Mf) and from two other points to detect the propagation of epileptiform discharges through the whole cortical surface (see insert in Fig. 2). The recording electrodes were connected to an 8 channel electroencephalograph (EEG) with a low frequency filter at 0.1 Hz and high frequency filter at 70 Hz. The ECoG was stored on computer memory by the aid of Digidata 1200B (BD, BNC, Axon Instruments, Inc.). For further details of the method see in Ref. 24.

In different groups of the animals (n = 7 in all groups) the somato-sensory cortical surface was locally pretreated with AOAA solutions of low concentrations (from 1–100 μM) or high concentrations (from 0.1–1 M) before the induction of epileptiform activity in order to test the concentration-dependency of the effect of AOAA. To investigate the time-dependency of its effect, the duration of the pretreatment period varied between 1 and 3 hours.

AOAA was dissolved in distilled water; its acidic pH was neutralized with 0.1 N NaOH, and was applied to the cortical surface by the aid of 2×2 mm filter paper, soaked with the solution. As representative of the effects of AOAA at low and high concentrations, we chose the parameters measured under the influence of 100 μM and 1M AOAA, respectively. In control animals the cortical surface was pretreated by physiological solution and the following epileptiform activity induced by 4-AP was considered as control seizure activity.

Electrical stimulation (3 V, 0.3 ms, 0.3 Hz square pulses) was delivered at the left whisker field through a bipolar needle electrode and the related evoked responses were recorded from the punctum maximum of right side somato-sensory cortical surface. This cortical point was chosen later to induce Pf. Ten individual evoked responses were averaged: the latency (measured from the rising phase of the stimulus artifact) and the peak amplitudes (measured from the baseline) of the averaged curve were assessed.

The effects of AOAA on the initiation and manifestation of seizure activity were investigated by measuring the latency of the first ictal period, the numbers and durations of ictal periods, the probability of seizure propagation as well as by analyzing the pattern (frequency and amplitude) of epileptiform discharges. Summated ictal activity, as an indicator of seizure susceptibility, was determined by multiplying of the numbers with the duration of individual ictal periods measured during 60 min.

Statistical analysis of stored data was carried out by Mathcad and Origin processing software completed by a homemade statistical program. Different parameters of control and AOAA-treated animals were compared and the significant differences (criterion: $P < 0.02$) were determined by Student's *t*-test.

During experiments, the general state (level of anesthesia, body temperature) of the rats was regularly checked. All procedures were conducted in accordance with the Guidelines for the Care and use for Laboratory Animals and the policy on the animal experiments of the American Physiological Society. 4-AP and AOAA were purchased from Sigma.

# RESULTS

## *Basic electrical and epileptiform activity of the cortex under the influence of 4-AP*

Evoked potentials recorded from untreated cortical surface were composed of three characteristic peaks (Fig. 1, Table 1). The dominant positive component of the response ($P_1$) was preceded by two additional negative components ($N_1$, $N_2$) of smaller amplitudes. Under the influence of 4-AP the configuration of the evoked responses showed a rapid and characteristic modification (Fig. 1A). While the $P_1$ peak – after a short transient increase – was completely abolished, the $N_1$ significantly increased and became the main component of the responses evoked by the same stimuli as in the controls (Table 1). The second negative peak of the responses recorded under the influence of 4-AP might correspond to the $N_2$ component of the control curve. Generally the evoked responses were very similar in appearance to the epileptiform discharges occurring spontaneously during ictus (not shown here).

The ECoG pattern of 4-AP-induced seizure activity has already been described together with the accompanying intracellular events both in the primary and mirror foci in anesthetized cats and rats [24, 25]. Shortly after the application of 4-AP, ictal episode occurred at the Pf then repeated spontaneously. In 90% of the animals simi-
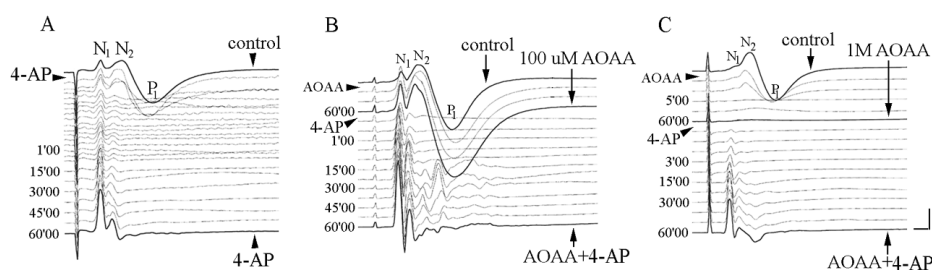
*Fig. 1.* Effect of low and high concentration of AOAA on the somato-sensory evoked responses. Averaged somato-sensory evoked responses recorded in different conditions. $N_1$, $N_2$: negative peaks, $P_1$: positive peaks. Negativity upward. A) Evoked potential recorded from untreated cortical surface and during 60 min of 4-AP application. B) Recordings from another animal in control condition then under the influence of AOAA of low concentration, followed by 60 min of 4-AP application. C) Evoked responses from the third animal in control condition then under the influence of AOAA of high concentration, followed by 60 min of 4-AP application. Calibration: 2.5 ms; 0.5 mV

lar synchronous paroxysmal activity developed at the Mf (Fig. 2B). Roughly, three different patterns of epileptiform discharges within the ictal periods could be distinguished according to their frequencies, amplitudes and waveforms. Most commonly, the seizure began with rapid, repetitive spikes of high frequencies (10–15 Hz) with small amplitudes, followed by repetitive spikes of 4–9 Hz or 1–3 Hz of spike-wave complex with higher amplitudes (Fig. 2).

*Table 1*
Parameters of different components of evoked responses

|  | Latency (ms) | | | Amplitude (mV) | | |
|---|---|---|---|---|---|---|
|  | $N_1$ | $N_2$ | $P_1$ | $N_1$ | $N_2$ | $P_1$ |
| Control (n = 7) | 4.9±0.1 | 8.3±0.18 | 13.55±0.35 | 0.23±0.1 | 0.37±0.11 | 1.12±0.35 |
| 4-AP | 4.7±0.07 | 6.7±0.12* | – | 1.055±0.07* | 0.34±0.08 | – |
| 100 µM AOAA (n = 7) | 4.8±0.15 | 7.1±0.25 | 14.1±0.3 | 0.7±0.11* | 0.7±0.18* | 1.7±0.34* |
| 100 µM AOAA + + 4-AP | 4.4±0.2 | 6.4±0.12* | – | 1.685±0.39* | 0.45±0.07 | – |
| 1M AOAA (n = 6) | – | – | – | – | – | – |
| 1M AOAA + 4-AP | 4.5±0.18 | 6.2±0.33* | – | 0.86±0.1* | 0.19±0.08 | – |

The mean ±SD values are represented. Stars indicate the significant differences, significance criterion: P ⌐ 0.02.
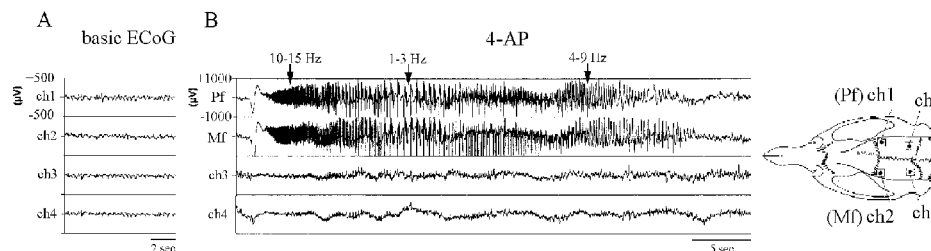
*Fig. 2.* Cortical ictal activity induced by 4-AP in anesthetized rat. A) Basic electrical activity of untreated cortex. B) ECoG patterns of 4-AP-induced cortical epileptiform activity 60 min after the application of 4-AP. The recording points are illustrated on the scheme. Ch 1, 2, 3, 4: channel 1, 2, 3, 4, respectively. Pf: Primary focus, Mf: Mirror focus

## The effects of AOAA at low concentrations on the physiological- and epileptiform cortical activity

Local pretreatment of the cortical surface with AOAA at low concentrations for 1 h did not influence the basic ECoG noticeably; neither induced spontaneous epileptiform discharges (Fig. 3B). The configurations of evoked responses did not modify dramatically, while the amplitudes of both $N_1$, $N_2$ and $P_1$ significantly increased without remarkable changes in their latencies (see the upper 4 lines in Fig. 1B, Table 1). However, 4-AP application, following pretreatment with AOAA characteristically and rapidly modified the shape and the amplitudes of the evoked potentials (Fig. 1B lower lines, Table 1), similarly as it was observed under the influence of 4-AP alone (Fig. 1A). The $P_1$ component of the evoked response rapidly disappeared. $N_1$ became the prominent component of the responses with significantly increased amplitude. It seemed as if the effects of AOAA and 4-AP were cumulative on the evoked responses.
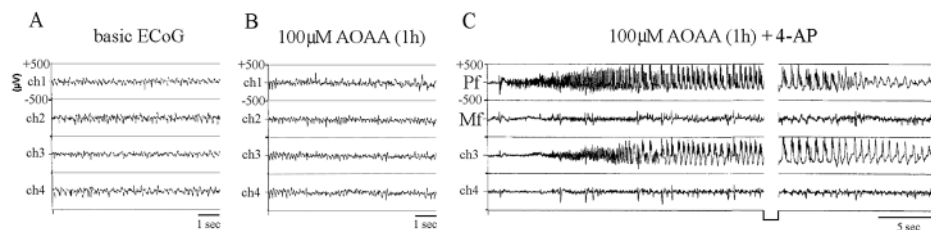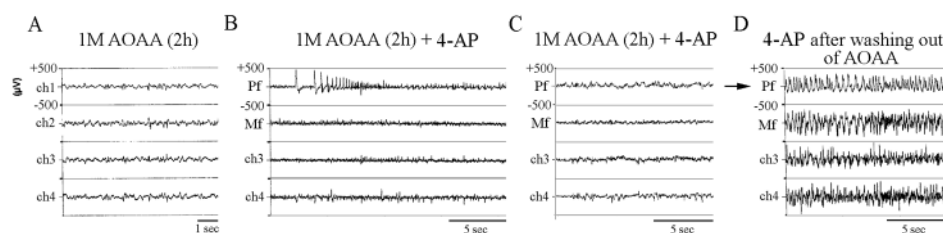


*Fig. 3.* Effects of AOAA at low concentration on the ECoG and the 4-AP-induced cortical epileptiform activity. A) Basic ECoG of untreated cortex. B) Basic ECoG after 1 h pretreatment with AOAA of low concentration. C) 4-AP-induced cortical epileptiform activity after pretreatment with AOAA. The initial and the terminating part of the ictal period can be seen; the break represents about 130 sec time window

Pretreatment with AOAA at low concentrations for 1 h period significantly facilitated the ipsilateral manifestation and propagation of epileptiform activity evoked by 4-AP (Fig. 3C). Although, the latency of the first ictal period and the numbers of the individual ictal periods did not change remarkably in the Pf, the ictal periods were significantly longer compared to controls resulting in a significantly longer summated ictal activity (Fig. 5A). The ratio of epileptiform discharges of different frequencies shifted in favor of spike-wave complex (1–3 Hz). In addition, a general decrease in the amplitudes of the epileptiform discharges was observed (Fig. 5C). Propagation of paroxysmal discharges on the ipsilateral hemisphere was highly facilitated, since synchronous seizure activity occurred on the whole ipsilateral hemisphere. However the spreading of epileptiform discharges to the contralateral hemisphere was strongly suppressed (Fig. 3C).

Since, pretreatment of the cortex with AOAA at low concentrations for longer periods (>2 h) influenced the induction, propagation and manifestation of epileptiform activity similarly, as it was described above for shorter treatment (1 h), it will not be considered here.

### The effects of AOAA at high concentrations on the physiological- and epileptiform cortical activity

The cortical pretreatment with AOAA at high concentrations did not modify the basic ECoG noticeably; neither induced spontaneous epileptiform discharges (Fig. 4A). However, during pretreatment, the amplitudes of evoked potentials rapidly and dramatically decreased (within the first 5 or 6 min), then the responses completely disappeared (Fig. 1C upper 6 lines). In spite of the complete depression of the evoked responses, the electrical stimulation of the whisker area with the same stimulus parameters gradually became effective again under the influence of 4-AP (Fig 1C lower lines). Recorded 60 min after 4-AP application the configuration of the responses



*Fig. 4.* Effects of AOAA of high concentration on the basic ECoG and on the 4-AP-induced seizure activity (A, B, C). See the significant suppression (B) or complete prevention (C) of seizure activity after 2 h pretreatment with AOAA. D) Recovered cortical seizure activity 30 min after removal of AOAA from the cortex, in continuous presence of 4-AP (in the same animal as of panel C)

became comparable to those, evoked under the influence of 4-AP in the absence of AOAA (compare Fig. 1C to 1A). In addition, the amplitudes and the latencies of $N_1$ and $N_2$ peaks approached the values of $N_1$ and $N_2$ peaks recorded in control animals in the presence of 4-AP alone (Table 1).

Pretreatment of the cortex with AOAA at high concentrations significantly suppressed epileptogenesis. The anticonvulsive feature of AOAA gradually reached its maximum at 2 h pretreatment (Fig. 5B). The pretreatment with AOAA of high concentrations completely prevented the induction of epileptiform activity in 43% of the animals (Fig. 4C). In the 57% of the animals the AOAA pretreatment did not block entirely but significantly delayed the initiation of the first ictal episode and strongly depressed the expression of seizure activity, which was restricted only to the Pf (Fig. 4B). The significant reductions in the numbers and the durations of ictal-like periods resulted in a highly reduced summated ictal activity as compared to the control value (Fig. 5B). The amplitudes of all characteristic epileptiform discharges decreased significantly (Fig. 5C). The short seizure periods consisted mostly of discharges of 1–9 Hz, while the discharges of higher frequencies were almost completely missing. Since, the propagation of the suppressed epileptiform activity to the contralateral
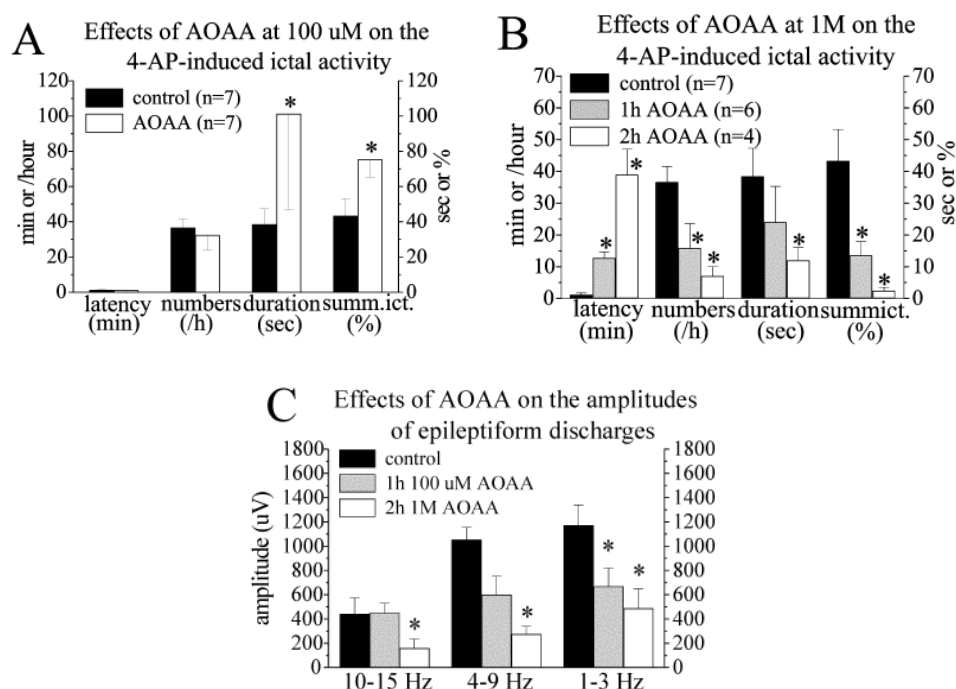


*Fig. 5.* Different values of 4-AP induced cortical seizure activity in control and AOAA-treated animals. In the case of AOAA of high concentration the data were collected from those treated animals, where some suppressed seizure activity was detectable (see in the text). Graphs represent the mean ±SD values. Stars indicate the significant differences, significance criterion: P ˂ 0.02

hemisphere was totally blocked, seizure activity did not manifest in the Mf in either of these animals. The inhibitory effect of AOAA at high concentrations turned out to be reversible. After removal of the drug by 10 min rinsing the cortical surface with warm saline, 4-AP was able again to induce seizure discharges comparable in appearance to the control seizure activity (Fig. 4D).

## DISCUSSION

In the present study the effect of AOAA on the cortical epileptogenicity, as well as on the basic ECoG and somato-sensory evoked responses was investigated in anesthetized rats. In the present work the facilitatory and inhibitory effect, as well as the concentration- and time-dependency of AOAA action was confirmed.

Based on our observations we conclude that local application of AOAA to the cortical surface apparently does not influence the basic cortical electric activity, nor does it induce spontaneous epileptiform discharges by itself at any tested concentrations. AOAA characteristically influences the somato-sensory evoked responses and the expression of 4-AP-induced cortical epileptogenicity in the same manner, depending on its concentration. (i) AOAA at low concentrations (in μM range) increases the amplitude of evoked responses and facilitates the expression and the ipsilateral propagation of cortical epileptiform discharges. (ii) AOAA at high concentrations (in M range) significantly suppresses the evoked potentials, as well as the initiation, manifestation and spreading of seizure discharges on both hemispheres. The inhibitory effect of AOAA at high concentrations is time-dependent and reversible.

AOAA was reported to induce spontaneous firing activity, increase evoked-potentials, cause hyperexcitability of pyramidal cells or to generate epileptiform discharges in the entorhinal cortex, hippocampus or cortical neurons *in vitro* [6, 21], and to provoke behavioral seizures *in vivo* [4, 6, 17, 21, 27, 28, 30]. Since, NMDA receptor antagonists prevent AOAA-induced neuronal lesions and seizures, it was suggested that the NMDA receptors dominantly involved in excitotoxic and proconvulsive effects of AOAA [17, 27, 29].

AOAA was shown to decrease the extracellular level of KYNA, the only known endogenous excitatory amino acid antagonist by inhibiting its synthesizing enzyme, the kynurenine-transaminase [7, 18, 23, 26, 29]. Insufficient neuroprotection and uncontrolled activation of NMDA receptors as a result of kynurenine-transaminase inhibition is supposed to be responsible for excitotoxicity and seizure induction [18, 29]. Wood et al. [31] reported that AOAA in 10–100 μM range also blocks the GABA-synthesizing enzyme glutamate-decarboxylase (GAD) and showed in experiments with synaptosomal preparations that the convulsive effect of AOAA is linearly associated with the significant decrease of GABA levels in nerve terminals. Reduced GAD activity was also found in some brain regions during AOAA-induced convulsions [29].

Based on these biochemical data we suppose that AOAA at low concentrations blocks the kynurenine-transaminase and GAD in our model as well. The consequen-

tial uncontrolled hyperactivity of NMDA receptors, in addition to the reduced GABA synthesis can be reasons of hyperexcitability, indicated by the increased somato-sensory evoked responses and the enhanced cortical seizure susceptibility in the present study. Furthermore, the impairment of intracellular energy metabolism [30] may also be involved in the detected facilitator effect of AOAA observed in our experiments.

In spite of the highly facilitated ipsilateral expression of paroxysmal discharges under the influence of AOAA at low concentrations, their propagation to the contralateral hemisphere was remarkably suppressed in our experiments. This phenomenon might be explained by the differences in the mechanisms and the neuronal networks how and where these discharges are propagated. The intrahemispherical propagation of discharges might happen through cortico-cortical connections between pyramidal neurons mediated preferentially by NMDA receptor [10], which can be overexcited under the influence of AOAA at low concentrations. On the other hand, the inter-hemispherical connecting pathways might operate with different neuro-transmitter- and receptor systems. It is also possible that hyperactivity of NMDA-receptors on GABAergic interneurons in the presence of AOAA at low concentrations can reinforce their inhibitory output selectively on those pyramidal cells, which project their axon collaterals to the contralateral hemisphere. This hypothesis is based on observations that some of GABAergic interneurons are able to operate with different functionally related groups of target neurons [9]. In addition, if we suppose that the enhanced inhibitory outputs terminate on those inhibitory interneurons, which normally control the propagation of seizure discharges around the focus, thus the indirect release of focal pyramidal cells from this inhibitory control can lead to facilitated ipsilateral seizure propagation. However, the selective inhibition of contralateral projecting neurons in the Pf can decrease the numbers of cells firing synchronously, that would be an explanation for smaller amplitudes of epileptiform discharges observed in these experiments.

Anticonvulsive action of AOAA has been reported on a wide variety of epilepsy models [11, 12, 14, 15]. The time- and concentration dependency of its anticonvulsive effect was also shown but in different epilepsy models [3, 8, 13].

AOAA under certain conditions blocks GABA-catabolizing enzyme (GABA-T) leading significant enhancement of intracellular GABA level in many brain regions [3, 15, 31]. Löscher et al. [16] reported that AOAA inhibits GABA-T without influencing the GABA-synthesizing enzyme (GAD) activity, suggesting that the inhibitory and anticonvulsive action of AOAA is due to rather the blockage of GABA degradation and not to the enhancement of GABA-synthesis. Wood et al. [32] also showed that the anticonvulsive effect of AOAA increases in parallel with the elevation of synaptosomal GABA contents through inhibition of GABA-degradation.

In addition, AOAA at μM range proved to be ineffective on GABA release from nerve terminals [20], however the long presence of AOAA at high concentrations (mM) was reported to facilitate GABA release and block GABA re-uptake, resulting the accumulation of GABA in the synaptic cleft [19, 22].

Based on these observations we suppose that in our experiments AOAA at high concentrations leads to a significant intra- and extracellular GABA accumulation

mainly by the inhibition of GABA-degradation, as well as by influencing the GABA release- and GABA-transport system. These cumulative effects of AOAA can provide an effective cortical inhibition and explain the reduced evoked responses, the weaker or the complete inhibition of seizure activity.

Since, the maximal anticonvulsive effect of AOAA developed after long pretreatment periods (>2 h), it is possible that for its most potent inhibitory effect a gradual increase of GABA content – up to a threshold level – is needed at the presynaptic terminals. This gradual increase of GABA level could explain the time-dependency of the anticonvulsive effect of AOAA.

Although AOAA has been reported to induce neuronal damage and cell death [5, 6, 29] we suppose that the inhibitory effect of AOAA is probably not the consequence of cell loss induced by AOAA, because of: (i) the lack of noticeable changes in the basic ECoG during the AOAA treatment; (ii) the reversibility of its anticonvulsive effect; in addition, (iii) evoked responses, under the influence of 4-AP after pretreatment with high concentrations of AOAA are similar in appearance to those, which can be seen in the presence of 4-AP alone. However, some neuronal loss cannot be excluded at the site of AOAA treatment. Nevertheless, if we consider some degree of cell loss in our experiments under the influence of AOAA, this could not be the explanation for the suppressed epileptogenicity. It is well demonstrated both in humans and in animal models that hippocampal sclerosis, which is a morphological modification in temporal lobe epilepsy, is characterized by excessive cell loss [2, 43]. Nevertheless, the severely damaged structure is highly epileptogenic and is capable for massive synchronous rhythmic seizure activity [1, 33].

In the intact brain the physiological balance of the excitation and inhibition is regulated by different controlling mechanisms in a relatively narrow operating range [2, 34]. In abnormal conditions, like in epilepsy, these controlling mechanisms can lose their efficacy and contribute to the development of an abnormal state of the brain [34]. This can be the reason why AOAA at high concentrations completely blocked the somato-sensory evoked responses but was not able to prevent the induction of epileptic-like responses under the influence of 4-AP (Fig. 1C). Therefore, we conclude that the effect of AOAA on the cortical activity depends not only on its concentration or the duration of its presence, but also on the actual state of the nervous system.

In summary we can conclude, that AOAA has concentration- and time-dependent effects on the 4-AP epilepsy model, *in vivo*. AOAA at low concentrations seems to be proconvulsive, probably as a consequence of over-activated NMDA receptors and a reduced GABA-synthesis. On the other hand, AOAA at high concentrations proved to be anticonvulsive by the possible blocking of GABA-degradation at the presynaptic side together with the increased GABA level in the synaptic cleft. The anticonvulsive effect of AOAA at high concentrations shows time-dependency and reversibility.

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