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# Non-NMDA receptor antagonist GYKI 52466 suppresses cortical afterdischarges in immature rats

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**Abstract**

GYKI 52466 (1-(4-aminophenyl)-4-methyl-7,8-methylendioxy-5H-2,3-benzo-diazepine), a non-competitive non-NMDA receptor antagonist, was tested against epileptic afterdischarges elicited by cortical stimulation in 12-, 18- and 25-day-old rats with implanted electrodes. Shortening of afterdischarges and a decrease in intensity of clonic movements accompanying both stimulation and afterdischarges were induced by the 20 mg/kg dose of GYKI 52466 in 18- and 25-day-old animals, whereas 12-day-old rat pups exhibited only shortening of electroencephalographic afterdischarges. The 10 mg/kg dose of GYKI 52466 did not significantly change afterdischarges in any age group. Motor skills were compromised after the 20 mg/kg dose of GYKI 52466. This effect was again more marked in 18- and 25-day-old animals than in the youngest group. In addition, anxiolytic-like action was observed in the jumping down test in 25-day-old rats. This effect was not influenced by a benzodiazepine antagonist flumazenil. On the contrary, the anticonvulsant action of GYKI 52466 was partly blocked by flumazenil, indicating thus multiple mechanisms of action of GYKI 52466. © 1997 Elsevier Science B.V.

*Keywords:* GYKI 52466 (1-(4-aminophenyl)-4-methyl-7,8-methylendioxy-5H-2,3-benzo-diazepine); Excitatory amino acid; Anticonvulsant action; Motor system; Flumazenil

## 1. Introduction

Suppression of excessive synaptic transmission mediated by excitatory amino acids represents one of the possible mechanisms of anticonvulsant action (Macdonald and Kelly, 1993). Until recently, the attention was focused only on antagonists of *N*-methyl-D-aspartate (NMDA) type of receptors due to an absence of specific antagonists of other, non-NMDA types of receptors (Honoré, 1991). The studies of the role of non-NMDA (AMPA/kainate) receptors in epilepsy and the possible use of their antagonists as anti-epileptics started in the early nineties, when two in vivo active, selective AMPA ($\alpha$-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor antagonists were described, a competitive antagonist NBQX (2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline (Sheardown et al., 1990)) and a noncompetitive one GYKI 52466 (1-(4-aminophenyl)-4-methyl-7,8-methylendioxy-5*H*-2,3-benzo-diazepine (Tarnawa et al., 1990)), and their anticonvulsant action was immediately demonstrated (Chapman, 1991).

GYKI 52466 was found to be effective in different models of epilepsy, although, only in doses impairing motor function (Berzsenyi et al., 1988; Tarnawa et al., 1989; Chapman et al., 1991; Smith et al., 1991; Meldrum et al., 1992; Yamaguchi et al., 1993; Dürmüller et al., 1994). All results with this drug were obtained using adult, fully mature animals and developmental data are missing. Such data are important from two points of view: (1) the majority of human epilepsies starts during infancy and childhood and immature brain is more prone to generate seizures (Freeman, 1995) and (2) overexpression of non-NMDA receptors was described during postnatal development in rats when compared with adult animals (Miller et al., 1990) as well as a higher sensitivity of immature brain to non-NMDA receptor agonists kainate and AMPA (Schoepp et al., 1990; McDonald and Johnston, 1990). Therefore we started to test the action of this non-NMDA receptor antagonist in immature rats in vivo using a model of epileptic afterdischarges, elicited by electrical stimulation of sensorimotor cortex. This model allows the evalua-

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tion of three different phenomena: movements accompanying stimulation induced by a direct activation of the motor system, EEG afterdischarges generated in the cerebral cortex and clonic seizures accompanying afterdischarge as a consequence of spread of epileptic activity from the cerebral cortex to brainstem and spinal motoneurons (Mareš et al., 1992; Kubová et al., 1993). Because of data on the impairment of motor functions in adult animals (Berzsenyi et al., 1988; Tarnawa et al., 1989; Chapman et al., 1991; Smith et al., 1991; Meldrum et al., 1992; Yamaguchi et al., 1993; Dürmüller et al., 1994) we performed the second experiment where the motor skills of rat pups were tested in identical age groups, using the dose of GYKI 52466 which was effective in the electrophysiological study.

GYKI 52466 is thought to act via a benzodiazepine binding site associated with the AMPA type glutamate receptors (Zorumski et al., 1993). Electrophysiological (Tarnawa et al., 1990; Ouardouz and Durand, 1991; Tarnawa et al., 1992), as well as whole-cell voltage-clamp studies (Donevan and Rogawski, 1993) confirmed that GYKI 52466 is a highly selective AMPA/kainate receptor antagonist which does not interact with NMDA, metabotropic glutamate or $GABA_A$ receptor-mediated responses. In contrast to another 2,3-benzodiazepine, tofisopam, GYKI 52466 exhibited some pharmacodynamic characteristics, resembling those of 1,4-benzodiazepines such as diazepam (e.g., anticonvulsant and muscle relaxant action (Rogawski, 1993)). Most effects of 1,4-benzodiazepines are due to selective interaction with 'central' benzodiazepine binding sites at $GABA_A$ receptor–chloride ionophore complex (Haefely, 1989). Tarnawa et al. (1989) reported that GYKI 52466 has some affinity to these 'central' benzodiazepine receptors, although much weaker than diazepam. The role of the interaction of GYKI 52466 with 'central' benzodiazepine receptors in anticonvulsant and sedative activity as well as in motor impairment is not yet clear. Block and Schwarz (1994) documented that the depressant effect of GYKI 52466 on spinal reflexes can be partially blocked by flumazenil, an antagonist of 'central' benzodiazepine receptors. These results suggest that at least part of the effects of GYKI 52466 are mediated through its interaction with $GABA_A$ receptors. In contrast, Löscher and Hönack (1994) determined anticonvulsant activity of GYKI 52466 and its alteration by flumazenil using seizure threshold tests for different generalized seizures. They reported that flumazenil failed to alter the anticonvulsant effects of GYKI 52466 in chosen models of seizures. Controversy of these results was an additional reason of our study; due to the current results only 25-day-old rats were used for this analysis.

## 2. Materials and Methods

Male and female offspring of Wistar rats were raised in the breeding station of our institute and kept at standard conditions. They received a standard diet and water at libitum. On the day of birth each litter was culled to 8 pups. The day of birth was counted as zero, all three age groups used (12, 18 and 25 days old) were preweanling ones. The experiments were performed according to the Animal Protection Law of the Czech Republic under approval of the Ethical Committee of the Institute of Physiology of the Academy of Sciences.

### *2.1. Epileptic afterdischarges*

This series was performed on 99 male Wistar albino rats in three age groups. Surgical preparation was made under ether anesthesia: two flat silver epidural stimulation electrodes were placed over the right sensorimotor area at coordinates AP = +1 and −1; $L = 2$ mm in relation to bregma; three similar recording electrodes over left frontal, sensorimotor area (AP = 0; $L = 2$ mm) and right and left occipital, visual areas (AP = 6; $L = 4$ in 25-day-old rats). The coordinates for younger age groups were recalculated according to the bregma–lambda distance. An indifferent electrode was localized in the nasal bone. All electrodes were fixed to the skull by means of fast curing dental acrylic, the animals were allowed to recover for at least 1 h, then their reflexes (righting, placing and suckling) were examined. The animals were offered to suck a 5% solution of glucose and then the experiments started.

The stimulation series lasting 15 s consisted of 1 ms rectangular biphasic pulses delivered at an 8 Hz frequency. The intensity of the stimulation current ranged from 2.5 to 4 mA, i.e., it was always higher than the threshold value (Makal et al., 1993). The stimulation series were repeated four times at 20 min intervals. The experimental animals were injected with a freshly prepared solution of GYKI 52466, 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine, (2 mg in 1 ml of physiological saline) at doses of 10 or 20 mg/kg intraperitoneally, 10 min after the end of the first afterdischarge. Two control groups were formed: intact rats and animals injected with the amount of physiological saline corresponding to the higher dose of GYKI 52466 (i.e., 10 ml/kg i.p.). Each age and dose group consisted of eight rats (exceptionally nine or ten).

Throughout the experiment the animals were in a plexiglass chamber and the electroencephalographic (EEG) recording started 2 min before the first stimulation, continued during the stimulation and afterdischarge and was interrupted 1 min after the end of afterdischarge. The only change with the second and further stimulations was the shorter recording before stimulation. The behavior of the animals during the EEG recording was marked directly on the EEG paper. Movements accompanying stimulation and afterdischarge were quantified by means of a five-point scale (Table 1). It was based on Racine's scale for cortical kindling experiments (Racine, 1972) modified only in point 1 and then an average score for each group was

Table 1
Scale for quantification of motor activity

| | |
|---|---|
| 0 | no movement at all |
| 1 | movements not synchronous with stimuli or spikes |
| 2 | head jerks synchronous with stimuli or sharp EEG graphoelements |
| 3 | clonic movements of forelimbs |
| 4 | clonic movements of forelimbs + rearing |
| 5 | clonic movements of forelimbs + rearing + falling |

calculated. The incidence and duration of afterdischarges as well as their EEG pattern were evaluated and a mean duration was computed. Frequency of spike-and-wave rhythm or rhythmic sharp waves was counted in all afterdischarges.

In order to obtain data on the alteration of anticonvulsant effects of GYKI 52466 by flumazenil, two additional groups of 25-day-old rats were used. Each of them consisted of at least eight animals. The surgery and stimulation protocol were the same as described above. One experimental group received an injection of flumazenil (freshly prepared solution in physiological solution with one drop of Tween 80 with a concentration of the drug 5 mg in 1 ml) at a dose of 5 mg/kg i.p. 5 min before administration of GYKI 52466 (20 mg/kg), i.e., 5 min after the end of the first afterdischarge. The timing of injections was based on data on a short half life of flumazenil in rats (Mandema et al., 1991). The dose of flumazenil used was previously shown to antagonize behavioral, anticonvulsant and muscle relaxant effect of benzodiazepines (Bonetti et al., 1982). The effects of flumazenil itself in this model were controlled in the second group where injection of GYKI 52466 was omitted.

Statistical analysis of the data was performed by means of BMDP programs (Dixon, 1990). The incidence of afterdischarges was compared by means of Fisher's exact test. The duration of afterdischarges was evaluated using the analysis of variance models. There were two grouping factors, age (with three levels) and dose (with four levels), and one repeated measures factor, repeated measurement of afterdischarge at each level of the age and dose. Logarithmic transformation was used to stabilize variance in the cells (Box and Cox, 1964). The complex revealed the presence of interactions and so restricted two-way and one-way analysis with multiple comparison methods were applied (Dixon, 1990; Winer, 1971; Holm, 1979). The frequency of EEG graphoelements was evaluated in the same manner as the duration of afterdischarges. Friedman's and Kruskal–Wallis nonparametric analysis with multiple comparison tests (Dixon, 1990) were used to evaluate behavioral scores. The level of statistical significance was set at 5%.

### 2.2. Motor skills

This series of experiments was performed in 152 rats. There were again three age groups, 12, 18 and 25 days old each divided into control (injected with physiological saline) and GYKI 52466 pretreated rats (20 mg/kg i.p.). Each age group consisted from 24–27 control as well as treated rats, i.e., there were pups from six litters in all groups, each litter was divided into experimental and control animals to minimize the variability of results. Testing started 5 min after application of the drug.

Seven neuro-behavioral tests chosen from the battery described by Altman and Sudarshan (1975) were slightly modified and then used to test the motor skills. Four tests (surface righting, negative geotaxis, bar holding and wire mesh) were common for all age groups. In addition, there were three tests adequate for individual age groups, the cliff avoidance test for 12-day-old pups, traversing bridge test for 18-day-old pups and jumping down (with choice) for 25-day-old pups.

Surface righting reflex: The pups were placed on a laboratory table in a supine position, 3 trials up to 60 s were evaluated. Time of righting and consistent placement of hindlimbs along the abdomen was recorded.

Geotactic reactions: The pups were placed on an inclined rough surface (30°) with the head facing downward (0°). The ability of pups to turn to 180° was recorded as well as the duration of the turn. The animals were tested for 90 s maximally.

Bar holding: The pups were held so that the forepaws touched a 25 cm long wooden rod with a diameter of 1 cm, which was hanging 25 cm above a padded surface. Time of grasping the bar with forelimbs and hindlimbs was recorded up to 120 s.

Wire mesh ascending: A surface consisting of 10 mm wire mesh, 45 cm high and 15 cm wide was placed at an angle of 70° in contact with a platform on the top and with an edge of the laboratory table at the bottom. To promote the ascending of rat pups their littermates were placed on the platform and individual rats were placed at the bottom of the wire mesh. Time of ascending for rejoining the siblings was recorded up to 120 s.

Cliff avoidance: The pups were placed on the smooth platform so that the forelimbs and the nose were lying on the edge of the platform. Time intervals before the animals turn totally away from the edge or before they fell down were measured. Time limit for this test was 30 s.

Crossing path: There were two elevated platforms (start and goal) connected by a plywood bridge (30 cm long and 3 cm wide). A litter of animals was placed on the goal platform and one pup at a time was removed and placed on the start platform. We recorded the time spent on the bridge to join littermates or to fall up to 120 s.

Jumping down (with choice): The pup was put on a platform that was placed 30 cm above the laboratory table. There were two cages, one with siblings and another empty, localized under the platform. Time interval to jumping down into one of the cages was measured. Maximal duration of the test was 120 s.

In the tests of bar holding, wire-mesh, cliff avoidance

and crossing the bridge, a box with a soft cover at the base served as a protection for the falling pups.

The data were analyzed with the Mann–Whitney rank sum test. The level of statistical significance was set again to 5%.

Similar to the first part of the study, the role of benzodiazepine receptors in the motor impairment induced by GYKI 52 466 was studied in 25-day-old rats using flumazenil. Experimental animals ($n = 20$) were pretreated with flumazenil at a dose of 5 mg/kg i.p. and 10 min later with GYKI 52 466 (20 mg/kg i.p.). A control group received flumazenil alone ($n = 14$). Another group of rats injected only with GYKI 52 466 ($n = 10$) was examined simultaneously. The same tests and time schedule were used as documented above, only the wire mesh test was deleted because of negative results in the first experiment.

## 3. Results

### 3.1. Epileptic afterdischarges

The first stimulation induced an epileptic afterdischarge in all animals. The stimulation always elicited clonic movements of head and forelimbs synchronous with stimuli, in some cases rearing and falling were observed. The afterdischarges were formed by spike-and-wave activity in 18- and 25-day-old rats and by rhythmic sharp delta waves in 12-day-old rat pups (Fig. 1). The frequency of the spike-and-wave rhythm was about 3/s, sharp waves in 12-day-old rat pups were repeated at a frequency of about 2/s. Afterdischarges were accompanied by clonic movements of the head and forelimbs which were synchronous with sharp elements in the afterdischarge. The motor pattern of these clonic seizures was practically the same as that of stimulus-bound movements, only their frequency was slower. Their intensity never exceeded the intensity of movements accompanying stimulation.

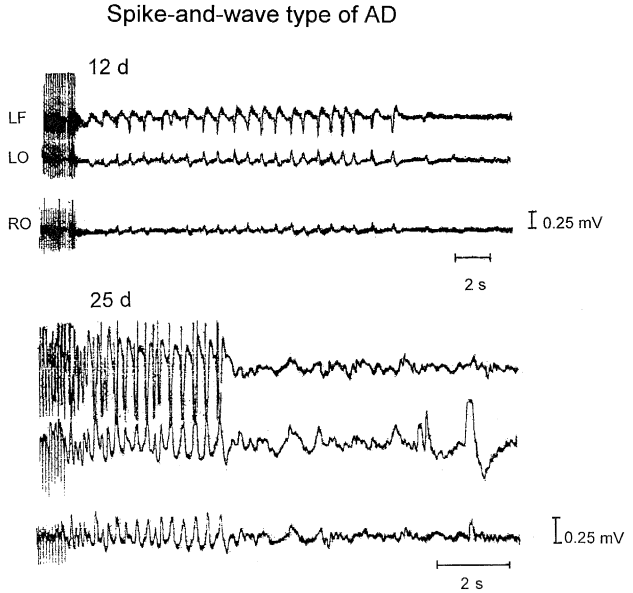


Fig. 1. Cortical afterdischarges in a 12- (upper part) and 25-day-old rat (lower part). The three leads in both recordings from top to bottom: LF = left frontal, RO = right occipital, LO = left occipital cortical area in reference connection. At the beginning of recordings are artifacts from the last stimuli of the stimulation series. Time mark, 2 s; amplitude calibration, 0.25 mV. Note different time scale in both recordings.

#### 3.1.1. Duration of afterdischarges

There were no significant differences in the duration of the first afterdischarge among experimental groups in any age. Therefore the relative duration, where the duration of the first afterdischarge was taken as 100%, was used for graphs. Under control conditions (untreated or physiological saline-treated rats) there was a tendency to an increase in duration of afterdischarges with repeated stimulations in all groups but 25-day-old untreated rats. Statistical significance was reached only for the fourth afterdischarge in 18-day-old untreated controls and in all age groups of saline-treated controls. Administration of GYKI 52466 in the dose of 10 mg/kg did not significantly change the duration of repeated afterdischarges in any age group. In contrast, the 20 mg/kg dose led to a significant shortening or blockade of the second and the third afterdischarge in 18-day-old rats and of the second afterdischarge in 25-day-old animals when compared to the appropriate first, predrug afterdischarge (Fig. 2). In 18-day-old animals, afterdischarges were abolished in 25 and 12.5% of animals after the second and third stimulation, respectively. In 25-day-old rats, the incidence of afterdischarges was significantly decreased after a second stimulation (they failed to appear in 62.5% of rats). The lengthening of afterdischarges with repeated stimulations seen in saline-treated controls was suppressed by the higher dose of GYKI 52466 in 12-day-old rats. If compared to the corresponding afterdischarges in the two control groups, the 20 mg/kg dose of GYKI 52466 led to a significant decrease in the duration of the second afterdischarge in 18- and 25-day-old rats, of the third afterdischarge in all age groups and of the fourth afterdischarge in 12- and 18-day-old rats.

Neither incidence nor duration of afterdischarges were altered in 25-day-old rats injected with flumazenil at a dose of 5 mg/kg compared to two control groups (Fig. 3). In contrast, flumazenil in the same dose reversed partially the suppressant effects of a 20 mg/kg dose of GYKI 52466. The duration of afterdischarge following a second stimulation was significantly longer in animals receiving the combination of both drugs compared to animals injected with a corresponding dose of GYKI 52466 itself. Also, pretreatment with flumazenil resulted in a significant increase of the incidence of afterdischarges after the second stimulation in animals receiving both drugs (87.5% versus 37.5% of rats given only GYKI 52466). No effects

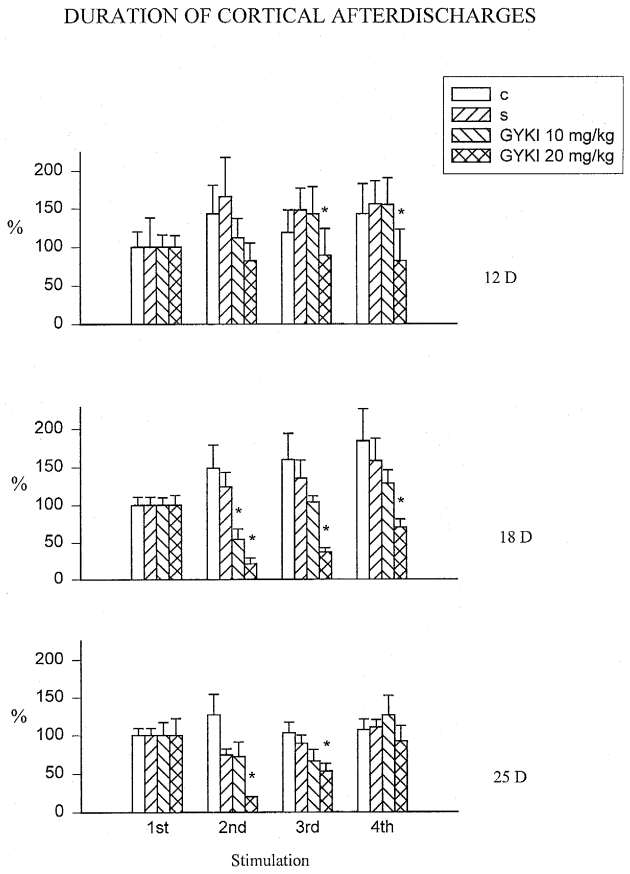


Fig. 2. Influence of GYKI 52466 on duration of afterdischarges (mean + S.E.M.) in rats 12, 18 and 25 days old (from top to bottom). Abscissa, four afterdischarges; ordinates, relative duration of afterdischarges; the mean duration of the first afterdischarge was always taken as 100%. Columns: c, intact controls; s, controls injected with physiological saline in a volume corresponding to higher dose of GYKI 52466; GYKI 10 mg/kg, rats injected after the first afterdischarge with GYKI 52466 in a dose of 10 mg/kg i.p.; GYKI 20 mg/kg, rats injected with the dose of 20 mg/kg. Significant differences in comparison with saline-injected animals are marked by asterisks.

of flumazenil on the suppressant activity of GYKI 52466 were found after a third and fourth stimulation.

### 3.1.2. Intensity of movements accompanying stimulation

In both control and 10 mg/kg groups the intensity of clonic movements did not significantly change with repeated stimulations. The intensity of movements accompanying the stimulation was significantly decreased only by the 20 mg/kg dose during the second stimulation in 18-day-old animals (compared to the first one). Comparison of the corresponding stimulation series among groups demonstrated that movements elicited by the second and third stimulation in 18- and 25-day-old groups were significantly less intense after the 20 mg/kg dose than in both control groups and the 10 mg/kg group (Fig. 4).

Flumazenil at 5 mg/kg exerted no effects on the intensity of movements induced directly by stimulation, but significantly increased the suppressant effects of 20 mg/kg GYKI 52466 after a second and third stimulation (data not shown).

### 3.1.3. Intensity of clonic seizures accompanying afterdischarges

Repeated afterdischarges resulted in less intense clonic seizures (when compared to the first, predrug afterdischarge) only after the 20 mg/kg dose in 18- and 25-day-old rats. A comparison of corresponding afterdischarges among individual groups demonstrated a decrease in seizure severity after the higher dose of GYKI 52466 for all postdrug afterdischarges in 18- and 25-day-old rats and after both doses of GYKI 52466 for the fourth afterdischarge only in the youngest group studied (Fig. 5).

Flumazenil did not alter the intensity of clonic seizures compared to corresponding controls. In contrast, pretreatment with flumazenil significantly reduced the suppressant effects of GYKI 52466 on the intensity of motor correlates of afterdischarges (data not shown).

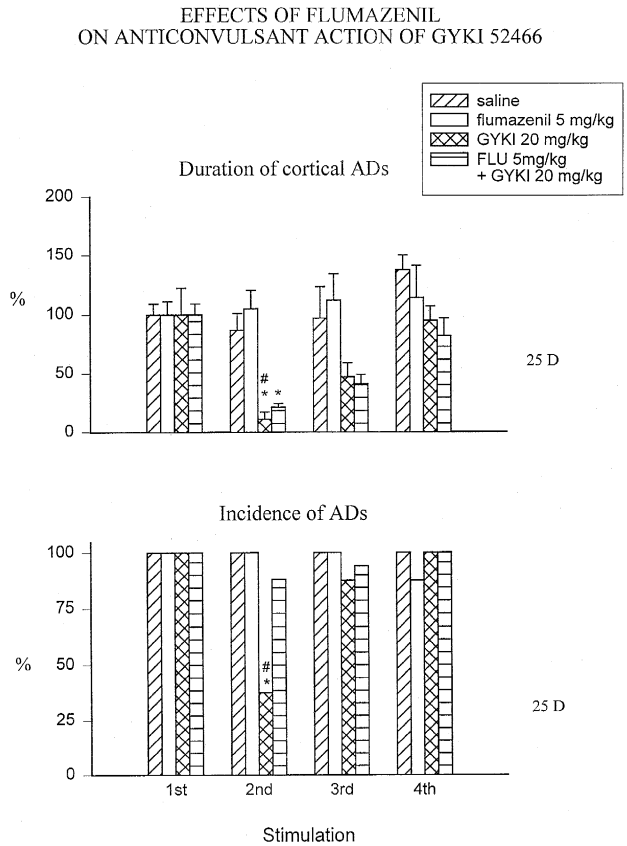


Fig. 3. Effect of GYKI 52466 and flumazenil on duration (upper part) and incidence (lower part) of afterdischagres in 25-day-old rats. Description of individual columns is in the inset (upper right). Other details as in Fig. 2, only ordinate in the lower graph denotes the percentage of animals generating afterdischarges and circle denotes significant difference against the combined GYKI 52466 and flumazenil group.

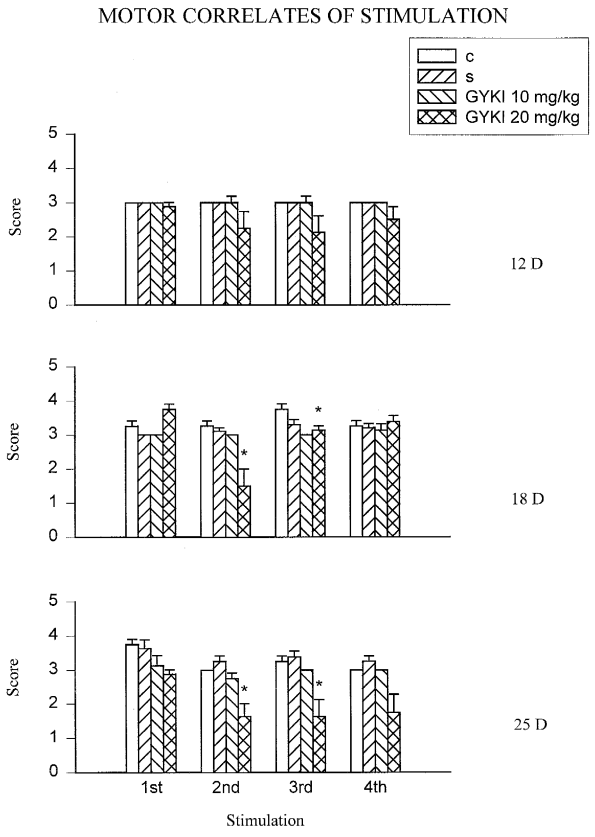


Fig. 4. Influence of GYKI 52466 on motor activity accompanying stimulation (mean score + S.E.M.). Details as in Fig. 4, only the ordinates, the five-point scale described in Table 1. Columns without S.E.M. denote groups where the intensity of movements was the same in all animals.

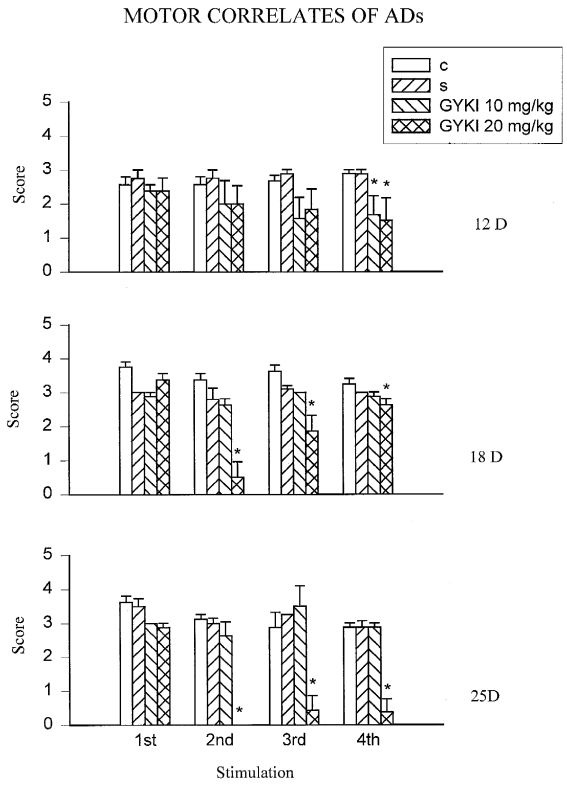


Fig. 5. Influence of GYKI 52466 on clonic seizures accompanying afterdischarges (mean score + S.E.M.). Details as in Fig. 2.

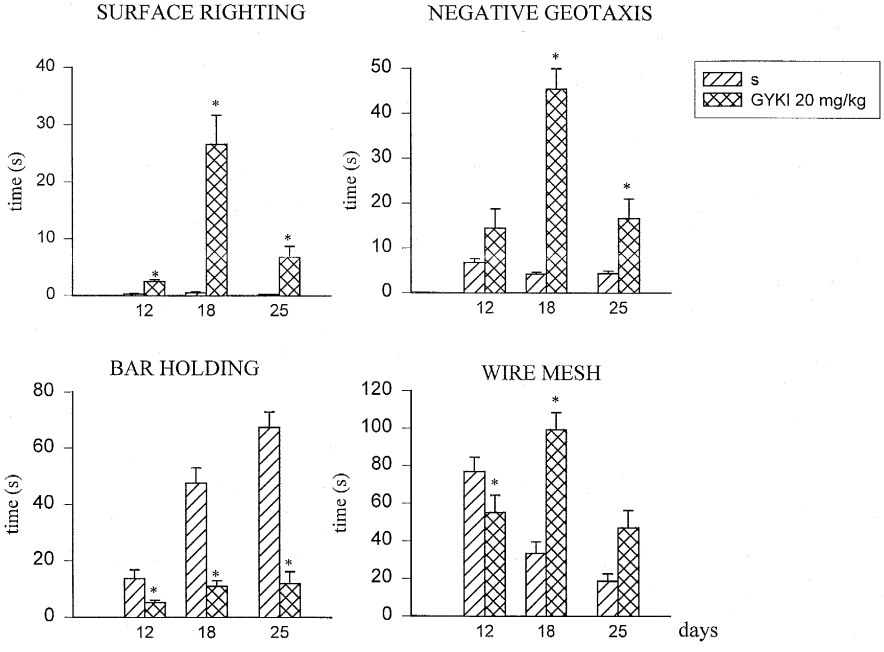


Fig. 6. Influence of GYKI 52466 on motor skills in three age groups of rats. Upper left, time necessary for surface righting; lower left, time of bar holding; upper right, latency of turning in the negative geotaxis test; lower right, time of ascending the wire mesh. In all graphs: abscissa, three age groups; ordinate, time in s. Columns: s, control groups (mean + S.E.M.); GYKI 20 mg/kg, rats pretreated with GYKI 52466. Asterisks mark significant differences.

### 3.2. Motor skills

GYKI 52466 at a dose of 20 mg/kg significantly increased the latency of turnings from supinal to normal position in all groups tested (Fig. 6).

In the negative geotaxis test, the latency of turning to 180°C was significantly prolonged only in 18- and 25-day-old rats, the difference in 12-day-old pups did not reach the level of statistical significance (Fig. 6).

In the bar holding test, all pretreated groups exhibited a significantly shortened time of grasping the bar with the forelimbs (Fig. 6). Signs of a hindlimb ataxia were observed, the animals were unable to pull up one or both hindlimbs to the bar.

GYKI 52466 significantly lengthened the latency of the ascending wire mesh in 18-day-old animals but the level of significance was not attained in 25-day-old rats. On the contrary, latency was significantly shortened in 12-day-old rat pups pretreated with GYKI 52466 (Fig. 6).

There was no difference between the control and pretreated group in the cliff avoidance test (Fig. 7).

GYKI 52466 significantly extended the time of bridge crossing to rejoin the littermates in 18-day-old rat pups (Fig. 7).

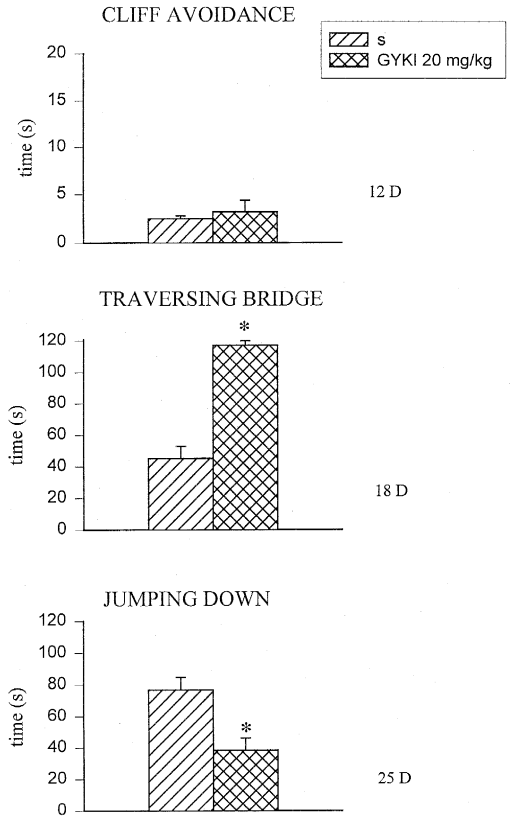


Fig. 7. Effects of GYKI 52466 in three tests specific for individual age groups. From top to bottom: cliff avoidance in 12-day-old rats; traversing bridge in 18-day-old rats; jumping down with choice in 25-day-old animals. Details as in Fig. 6.

In the jumping down test, all the tested young chose the home cage. GYKI 52466 significantly shortened the time to jumping in the group tested, i.e., 25 days old rats (Fig. 7). The same result was obtained in the second group of ten animals (controls to the action of flumazenil) a few weeks later.

Flumazenil alone has no effect on any of the four tests performed, the results were identical with those of the control rats. Pretreatment with flumazenil did not change the effects of GYKI 52466 in any of the four tests used (data not shown).

## 4. Discussion

The higher (20 mg/kg) dose of GYKI 52466 exhibited a marked anticonvulsant action in our experiments. It shortened the duration of afterdischarges and decreased the intensity of both stimulation-bound movements and clonic seizures accompanying afterdischarges. These changes were marked in 18- and 25-day-old rats, whereas the youngest group exhibited a significant shortening of afterdischarges, but the intensity of clonic movements only tended to decrease. Results similar to those found in our 18- and 25-day-old rats were published by Meldrum and coworkers in fully amygdala kindled adult animals. His model was more sensitive to GYKI 52466 because afterdischarges were shortened and the severity of motor seizures was decreased after both 10 and 20 mg/kg doses (Meldrum et al., 1992; Dürmüller et al., 1994). The change in the afterdischarge duration may signify that the generator of afterdischarges was influenced. Two processes playing a role in this action might be further differentiated: the generation of cortical afterdischarges and their progressive prolongation with repeated stimulations. The progressive prolongation of afterdischarges in our control groups may be taken as a sign of progressive epileptogenesis ('partial kindling', Racine et al., 1973) and it is usually more sensitive to the action of anticonvulsant drugs than the proper generation of afterdischarges (for review Sato et al., 1990; Engel, 1992). Different mechanisms of progressive prolongation and of generation of afterdischarges were suggested also by our results with benzodiazepines clonazepam and midazolam where low doses of these drugs blocked the progressive lengthening of afterdischarges leaving the duration of afterdischarge at the level of the first afterdischarge (Kubová et al., 1993). Similar results were obtained with ketamine (Kubová and Mareš, 1995). The present results with GYKI 52466 in 18- and 25-day-old rats (Fig. 2) might be taken as a sign that this non-NMDA antagonist is able to influence the generation of afterdischarges but progressive lengthening is not blocked, it only started from a lower level. Another possible explanation supported by the data of Mandema et al. (1991) is a short duration of the action of GYKI 52466 fading out even after 30 min, i.e., at the time of the third stimulation.

Movements elicited by stimulation represent an expression of the direct activation of motor system and they could be hardly influenced by antiepileptic drugs (Kubová et al., 1990, 1996). Only ketamine, a noncompetitive NMDA antagonist, was found to decrease the intensity of these movements in adult (Mareš et al., 1992) as well as developing rats (Kubová and Mareš, 1995). GYKI 52466 exhibited a similar action but with a little different developmental profile. Ketamine was active in rats 12 and 25 days old (Kubová and Mareš, 1995), whereas our present results demonstrated positive findings in all three age groups studied. A competitive AMPA antagonist NBQX exhibited effects similar to GYKI 52466 (Mareš et al., 1997). These data lead to a question about the role of excitatory amino acids in generation of these movements and in the motor system in general. Some tracts descending from the cerebral cortex (e.g., corticorubral and corticostriatal pathway) use excitatory amino acids as their transmitters (Headley and Grillner, 1990). The central muscle relaxant effect of GYKI 52466 and its inhibitory action on reflex transmission at the spinal segmental level were described (Tarnawa et al., 1989; Farkas and Ono, 1995). Therefore the action of this compound might be partly due to a direct influence of excitatory amino acid antagonists on the motor system.

Clonic seizures forming a motor correlate of afterdischarges could be more easily influenced by various drugs than movements accompanying stimulation. Among many anticonvulsants studied, until now phenobarbital, valproate and benzodiazepines clonazepam and midazolam were effective against this phenomenon (Kubová et al., 1993, 1996; Polášek et al., 1996). The activation of the motor system by epileptic activity arising in this system may not be so strong as the direct activation by the electrical stimulation of the motor cortex. There is an alternative explanation, motor correlates of afterdischarges may be taken as a sign of the spread of epileptic activity from an unknown generator outside the motor system into this system, i.e., the phenomenon probably based on the activation of multisynaptic pathways in contrast to the direct activation of the oligosynaptic motor system. The hypothesis that generation and spread of epileptic activity are based on different mechanisms was formulated by Woodbury (1972). He came to this conclusion because anticonvulsants could act differently on the two phenomena in question. In any case our data are in agreement with the recent findings on the action of GYKI 52466 on amygdala kindling (Dürmüller et al., 1994).

The dose of GYKI 52466 exhibiting anticonvulsant action in our model resulted also in disturbances of the motor system as was demonstrated in the majority of the tests used in 18- and 25-day-old rats. Our data are in agreement with findings describing ataxia after higher doses of GYKI 52466 in adult animals (Yamaguchi et al., 1993; Danysz et al., 1994). These effects may be due to a direct action of GYKI 52466 on the motor system which may also form a background for the attenuation of movements accompanying stimulation. The anatomical basis for this action is probably formed by excitatory amino acidergic pathways in the motor system (Headley and Grillner, 1990).

Non-NMDA receptors are present in the rat brain at very early stages of ontogeny (Insel et al., 1990); the flip type of AMPA receptors being present at birth and the flop form of these receptors since the 14th postnatal day in rats (Monyer et al., 1991). GYKI 52466 as a noncompetitive antagonist at AMPA/kainate receptor (Donevan and Rogawski, 1993) acts by a novel allosteric mechanism (Zorumski et al., 1993). Although the development of the AMPA receptor was studied (McDonald and Johnston, 1990; Shaw and Lanius, 1992), unfortunately, no data are available for the development of this novel binding site for GYKI 52466. Our results suggest that this binding site is present but probably not yet fully mature in the youngest group studied (12 days old) and it might reach full capacity at the age of 18 days. In connection with possible advantages of noncompetitive over competitive non-NMDA antagonists as concerns anticonvulsant activity (Yamaguchi et al., 1993), GYKI 52466 and its congeners deserve to be studied in other models of seizures in adult as well as developing animals, but unwanted side effects have to be taken into account.

Shortening of the latency to jump in the jumping down test in 25-day-old rats may be taken as an anxiolytic action of GYKI 52466. This surprising result was repeated in another group (see Sections 2 and 3). Such an effect might be due to the interaction of GYKI 52466 with benzodiazepine receptors as suggested by Block and Schwarz (1994). Therefore, the experiments with flumazenil were performed in this age group to confirm or exclude possible participation of benzodiazepine receptors in both anxiolytic and anticonvulsant action of GYKI 52466.

The imidazodiazepine flumazenil (Ro 15-1788) was initially characterized as a benzodiazepine receptor antagonist without intrinsic activity (Hunkeler et al., 1981). Later on however, partial benzodiazepine agonistic properties were documented (Dantzer and Pério, 1982). In addition, we reported the stable anticonvulsant effects of high doses of flumazenil in a model of pentylenetetrazol-induced seizures during development of the rat (Rathouská et al., 1993). In the present study, flumazenil was used in such a dose, which did not exhibit any effect in a model of cortical afterdischarges when used alone but which was previously documented to block the effects of 1,4-benzodiazepines (Hunkeler et al., 1981). In the present study, flumazenil was found to prevent some anticonvulsant effects of GYKI 52466. Duration of the second afterdischarge was significantly longer in animals receiving GYKI 52466 in combination with flumazenil than GYKI 52466 alone, however, in both experimental groups the afterdischarge duration was significantly shorter than in the corresponding controls. Also, premedication with flumazenil

returned the incidence of afterdischarges after the second stimulation to the control level.

Coadministration of flumazenil also affected the effect of GYKI 52466 on motor correlates accompanying both direct electrical stimulation of sensorimotor cortex and cortical afterdischarges. Flumazenil was found to decrease or abolish the suppressant effect of GYKI 52466 on the intensity of clonic movements accompanying afterdischarges. On the contrary, the effect of GYKI 52466 on direct activation of sensorimotor cortex was augmented by flumazenil. These opposite effects on different phenomena make a possibility of pharmacokinetic interaction due to a common route of administration highly improbable.

These results indicate that a part of the anticonvulsant effects of GYKI 52466 found in a model of cortical afterdischarges is mediated by flumazenil-sensitive benzodiazepine receptors. Similar results strongly supporting an idea that benzodiazepine receptors could participate in the effects of GYKI 52466 were published by Block and Schwarz (1994). They demonstrated that depressant action of GYKI 52466 on spinal reflex transmission is reduced by coadministration of flumazenil. Also, binding studies revealed that GYKI 52466 has some affinity towards the benzodiazepine receptor but weaker than that of diazepam (Tarnawa et al., 1989). In contrast, Löscher and Hönack (1994) did not find any changes of anticonvulsant effects of GYKI 52466 measured by thresholds for drug-induced motor convulsions after coadministration of flumazenil. These contradictory results might be explained by the different models of seizures used. Failure of flumazenil to antagonise motor impairment due to GYKI 52466 is in agreement with the data of Löscher and Hönack (1994). An anxiolytic effect of GYKI 52466 in the jumping down test deserves further analysis. On the basis of our present results we can only conclude that it was an active jumping without any hesitation seen in control animals. The lack of effect of flumazenil on this behavior led us to the conclusion that described anxiolytic activity of GYKI 52466 might be due to an interaction with AMPA receptors.

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## References

Altman, J., Sudarshan, K., 1975. Postnatal development of locomotion in the laboratory rat. Anim. Behav. 23, 896–920.

Berzsenyi, P., Tarnawa, I., Farkas, S., Andrási, F., 1988. Pharmacology of a new centrally acting muscle relaxant. Pharmacol. Res. Commun. 20 (Suppl. 1), 139–140.

Block, F., Schwarz, M., 1994. The depressant effect of GYKI 52466 on spinal reflex transmission in rats is mediated via non-NMDA and benzodiazepine receptors. Eur. J. Pharmacol. 256, 149–153.

Bonetti, E.P., Pieri, L., Cumin, R., Pieri, M., Gamzu, E.R., Muller, R.K.M., Haefely, W., 1982. Benzodiazepine antagonist Ro 15-1788: Neurological and behavioral effects. Psychopharmacology 78, 8–18.

Box, G.E.P., Cox, D.R., 1964. Analysis of transformations. J. R. Stat. Soc. Ser. B: 26, 211–252.

Chapman, A.G., 1991. Excitatory amino acid antagonists and therapy of epilepsy. In: Meldrum, B.S. (Ed.), Excitatory Amino Acid Antagonists. Blackwell Scientific Publications, London, pp. 265–286.

Chapman, A.G., Smith, S.E., Meldrum, B.S., 1991. The anticonvulsant effect of the non-NMDA antagonists, NBQX and GYKI 52466, in mice. Epilepsy Res. 9, 92–96.

Dantzer, R., Pério, A., 1982. Behavioural evidence for partial agonist properties of Ro 15-1788, a benzodiazepine receptor antagonist. Eur. J. Pharmacol. 81, 655–658.

Danysz, W., Essmann, U., Bresink, I., Wilke, R., 1994. Glutamate antagonists have different effects on spontaneous locomotor activity in rats. Pharmacol. Biochem. Behav. 48, 111–118.

Dixon, W.J. (Ed.), 1990. BMDP Statistical Software. UCLA Press, Los Angeles, CA.

Donevan, S.D., Rogawski, M.A., 1993. GYKI 52466, a 2,3-benzodiazepine, is a highly selective, noncompetitive antagonist of AMPA/kainate receptor responses. Neuron 10, 51–59.

Dürmüller, N., Craggs, M., Meldrum, B.S., 1994. The effect of non-NMDA antagonists GYKI 52466 and NBQX and the competitive NMDA receptor antagonist D-CPPene on the development of amygdala kindling and on amygdala-kindled seizures. Epilepsy Res. 17, 167–174.

Engel Jr., J., 1992. Experimental animal models of epilepsy: Classification and relevance to human epileptic phenomena. In: Avanzini, G., Engel Jr., J., Fariello, R., Heinemann, U. (Eds.), Neurotransmitters in Epilepsy. Elsevier, Amsterdam, pp. 9–20.

Farkas, S., Ono, H., 1995. Participation of NMDA and non-NMDA excitatory amino acid receptors in the mediation of spinal reflex potentials in rats: An in vivo study. Br. J. Pharmacol. 114, 1193–1205.

Freeman, J.M., 1995. A clinician's look at the developmental neurobiology of epilepsy. In: Schwartzkroin, P.A., Moshe, S.L., Noebels, J.L., Swann J.W. (Eds.), Brain Development and Epilepsy. Oxford University Press, New York, NY, pp. 9–33.

Haefely, W., 1989. Benzodiazepines: Mechanisms of action. In: Levy, R.H., Dreifuss, F.E., Mattson, R.H., Meldrum, B.S., Penry, J.K. (Eds.), Antiepileptic Drugs, 3rd ed. Raven Press, New York, NY, pp. 721–734.

Headley, P.M., Grillner, S., 1990. Excitatory amino acids and synaptic transmission: The evidence for a physiological function. Trends Pharmacol. Sci. 11, 205–211.

Holm, S., 1979. A simple sequentially rejective multiple test procedure. Scand. J. Stat. 6, 56–70.

Honoré, T., 1991. Inhibitors of kainate and AMPA ionophore receptors. In: Meldrum, B.S. (Ed.), Excitatory Amino Acid Antagonists. Blackwell, Oxford, pp. 180–194.

Hunkeler, W., Mohler, H., Pieri, L., Polc, P., Bonetti, E.P., Cumin, R., Schaffner, R., Haefely, W., 1981. Selective antagonists of benzodiazepines. Nature 290, 514–516.

Insel, T.R., Miller, L.P., Gelhard, R.E., 1990. The ontogeny of excitatory amino acid receptors in rat forebrain. I. *N*-methyl-D-aspartate and quisqualate receptors. Neuroscience 35, 31–43.

Kubová, H., Mareš, P., 1995. Suppression of cortical epileptic afterdischarges by ketamine is not stable during ontogenesis in rats. Pharmacol. Biochem. Behav. 52, 489–492.

Kubová, H., Makal, V., Miňová, M., Vaňková, S., Mareš, P., 1990. Influence of clonazepam on cortical epileptic afterdischarges in rats. Arch. Int. Pharmacodyn. 307, 49–59.

Kubová, H., Mareš, P., Vorlíček, J., 1993. Stable anticonvulsant action of benzodiazepines during development in rats. J. Pharm. Pharmacol. 45, 807–810.

Kubová, H., Mocková, M., Lanštiaková, M., Mareš, P., Vorlíček, J.,

1996. Pharmacology of cortical epileptic afterdischarges in rats. Epilepsia 37, 336–341.

Löscher, W., Hönack, D., 1994. Effects of the non-NMDA antagonists NBQX and the 2,3-benzodiazepine GYKI 52466 on different seizure types in mice: Comparison with diazepam and interactions with flumazenil. Br. J. Pharmacol. 113, 1349–1357.

Macdonald, R.L., Kelly, K.M., 1993. Antiepileptic drug mechanisms of action. Epilepsia 34 (Suppl. 5), S1–S8.

Makal, V., Miňová, M., Kubová, H., Mareš, P., 1993. Developmental changes of thresholds for cortical epileptic afterdischarges. Physiol. Res. 42, 49–52.

Mandema, J.W., Gubbens-Stibbe, J.N., Danhof, M., 1991. Stability and pharmacokinetics of flumazenil in the rat. Psychopharmacology 103, 384–387.

Mareš, P., Lanštiaková, M., Vaňková, S., Kubová, H., Velíšek, L., 1992. Ketamine blocks cortical epileptic afterdischarges but not paired-pulse and frequency potentiation. Neuroscience 50, 339–344.

Mareš, P., Mikulecká, A., Pometlová, M., 1997. Anticonvulsant action of NBQX in immature rats: Comparison with the effects on motor performance. J. Pharmacol. Exp. Ther., in press.

McDonald, J.W., Johnston, M.V., 1990. Physiological and pathophysiological roles of excitatory amino acids during central nervous system development. Brain Res. Rev. 15, 41–70.

Meldrum, B.S., Craggs, M.D., Dürmüller, N., Smith, S.E., Chapman, A.G., 1992. The effects of AMPA receptor antagonists on kindled seizures and on reflex epilepsy in rodents and primates. Epilepsy Res. 9 (Suppl.), 307–311.

Miller, L.P., Johnson, A.E., Gelhard, R.E., Insel, T.R., 1990. The ontogeny of excitatory amino acid receptors in the rat forebrain. II. Kainic acid receptors. Neuroscience 35, 45–51.

Monyer, H., Seeburg, P.H., Wisden, W., 1991. Glutamate-operated channels: Developmentally early and mature forms arise by alternative splicing. Neuron 6, 799–810.

Ouardouz, M., Durand, J., 1991. GYKI 52466 antagonizes glutamate responses but not NMDA and kainate responses in rat abducens motoneurones. Neurosci. Lett. 125, 5–8.

Polášek, R., Kubová, H., Šlamberová, R., Mareš, P., Vorlíček, J., 1996. Suppression of cortical epileptic afterdischarges in developing rats by anticonvulsants increasing GABAergic inhibition. Epilepsy Res. 25, 177–184.

Racine, R.J., 1972. Modification of seizure activity by electrical stimulation. II. Motor seizures. Electroencephalogr. Clin. Neurophysiol. 32, 281–294.

Racine, R.J., Burnham, W., Gartner, J., Levitan, D., 1973. Rates of motor seizure development in rats subjected to electrical brain stimulation: Strain and interstimulus interval effects. Electroencephalogr. Clin. Neurophysiol. 35, 553–556.

Rathouská, J., Kubová, H., Mareš, P., Vorlíček, J., 1993. Anticonvulsant activity of flumazenil in rats during ontogenetic development. Pharmacol. Biochem. Behav. 44, 581–586.

Rogawski, M.A., 1993. Therapeutic potential of excitatory amino acid antagonists: channel blockers and 2,3-benzodiazepines. Trends Pharmacol. Sci. 14, 325–331.

Sato, M., Racine, R.J., McIntyre, D.C., 1990. Kindling: Basic mechanisms and clinical validity. Electroencephalogr. Clin. Neurophysiol. 76, 459–472.

Schoepp, D.D., Gamble, A.Y., Salhoff, C.R., Johnson, B.G., Ornstein, P.L., 1990. Excitatory amino acid-induced convulsions in neonatal rats mediated by distinct receptor subtypes. Eur. J. Pharmacol. 182, 421–427.

Shaw, C., Lanius, R.A., 1992. Cortical AMPA receptors: Age-dependent regulation by cellular depolarization and agonist stimulation. Develop. Brain Res. 68, 225–231.

Sheardown, M.J., Nielsen, E.O., Hansen, A.J., Jacobsen, P., Honoré, T., 1990. 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(F)quinoxaline: A neuroprotectant for cerebral ischemia. Science 247, 571–574.

Smith, S.E., Dürmüller, N., Meldrum, B.S., 1991. The non-*N*-methyl-D-aspartate receptor antagonists, GYKI 52466 and NBQX are anticonvulsant in two animal models of reflex epilepsy. Eur. J. Pharmacol. 201, 179–183.

Tarnawa, I., Farkas, S., Berzsenyi, P., Pataki, A., Andrasi, F., 1989. Electrophysiological studies with a 2,3-benzodiazepine muscle relaxant: GYKI 52466. Eur. J. Pharmacol. 167, 193–199.

Tarnawa, I., Engberg, I., Flatman, J.A., 1990. GYKI 52466, an inhibitor of spinal reflexes is a potent quisqualate antagonist. In: Lubec, G., Rosenthal, G.A. (Eds.), Amino Acids: Chemistry, Biology and Medicine. ESCOM Science Publishers, Leiden, pp. 538–546.

Tarnawa, I., Molnar, P., Gaal, L., Andrasi, F., 1992. Inhibition of hippocampal field potentials by GYKI 52466 in vitro and in vivo. Acta Physiol. Hung. 79, 163–169.

Winer, B.J., 1971. Statistical Principles in Experimental Design. McGraw-Hill, New York, NY.

Woodbury, D.M., 1972. Applications to drug evaluations. In: Purpura, D.P., Penry, J.K., Tower, D., Woodbury, D.M., Walter, R. (Eds), Experimental Models of Epilepsy. Raven Press, New York, pp. 557–583.

Yamaguchi, S., Donevan, S.D., Rogawski, M.A., 1993. Anticonvulsant activity of AMPA/kainate antagonists: Comparison of GYKI 52466 and NBQX in maximal electroshock and chemoconvulsant seizure models. Epilepsy Res. 15, 179–184.

Zorumski, C.F., Yamada, K.A., Price, M.T., Olney, J.W., 1993. A benzodiazepine recognition site associated with the non-NMDA glutamate receptor. Neuron 10, 61–67.