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A. CZŁONKOWSKA [1], M. SIEMIĄTKOWSKI [1], A. PŁAŹNIK [1,2]

SOME BEHAVIORAL EFFECTS OF AMPA/KAINATE RECEPTOR AGONIST AND ANTAGONISTS

[1] Department of Experimental and Clinical Pharmacology, Medical Academy, Warsaw
[2] Department of Pharmacology and Physiology of the Nervous System, Institute of Psychiatry and Neurology, Warsaw, Poland

The effects of an agonist (S-AMPA, i.c.v.), as well as competitive (CNQX, NBQX, DNQX, i.p.), and noncompetitive antagonists (GYKI 52466, i.p.) at the AMPA/kainate receptors were examined in the open field and the Vogel tests of anxiety. It was found that both kinds of antagonists inhibited rat exploratory behavior in a dose-dependent manner, at the dose range exibiting a clear-cut tendency to decrease rat locomotor activity. They appeared inactive in the Vogel test over an examined dose-range. S-AMPA, whereas not changing in a significant way rat behavior in the open field, significantly enhanced the suppressive influence of a shock on drinking in the Vogel test. The drug administered at the dose of 2 μg/5 μl, i.c.v., revealed also a tendency to decrease the motor activity followed by prodromal symptoms of epileptic-like activity in some subjects. It is concluded that AMPA/kainate receptors probably are not directly involved in the control of rat emotional behavior. Thus, their primary role as putative neuroprotective and anticonvulsant agents is indirectly confirmed.

Key words: *S-AMPA, AMPA/kainate receptor antagonists, behavior, rats.*

INTRODUCTION

Benzodiazepine derivates though predominating in the therapy of anxiety disorders over the last three decades are not ideal agents, because of their side effects as well tolerance and dependence phenomena. Consequently, there is still a need for more selective and safer compounds.

In the brain an optimal physiological balance in regulating many central functions is achieved by the presence of opposing mechanisms involving excitatory (glutamate) and inhibitory (γ-aminobutyric acid, GABA) systems, respectively (1). Glutamate activates several types of metabotropic receptors and three major types of ionotropic receptor: NMDA, AMPA and kainate

receptors. The last two are sometimes still classified in one category of the non-NMDA receptors, as a result of the lack of receptor-selective drugs. It is known for example, that the consequences of enhancement of GABAergic transmission resemble to a certain extent those of inhibition of glutamate receptors (2). It appears true over a broad spectrum of brain activities, from regulating neuronal excitability and antiepileptic profile of action, to motor depression and anxiolysis (3—9). However, antagonists at the NMDA receptor, the best characterized type of the glutamate receptor, do not appear to offer advantages over presently known anxiolytic drugs because of their potency to develop memory and motor impairment as well as ataxia (1). On the other hand, in contrary to NMDA receptor ligands, our knowledge about compounds acting at AMPA/kainate receptors remains very limited. AMPA/kainate receptors can be regulated by drugs acting at different sites. Apart from the glutamate site to which competitive antagonists (e. g. NBQX, CNQX, DNQX) and agonists (AMPA, kainate) bind, there is also a site to which noncompetitive antagonists (e. g. GYKI 52466, GYKI 53655) show their affinity. Another binding site (blocked by synthetic or natural analogues of some spider and wasp toxins) is located within the ion channel itself (10)..

Considering all the aforementioned facts and the demand for more selective and safer anti-anxiety drugs, it seemed that the putative anxiolytic profile of action of AMPA/kainate competitive and noncompetitive antagonists deserved to be investigated. Interestingly, there are some preliminary data indicating involvement of AMPA/kainate receptors in the control of emotional behavior (22, 27). With this in mind we examined in the present experiment the effects of CNQX, NBQX, DNQX, GYKI 52466 and AMPA in two animal models of anxiety: the open field test and the Vogel test..

MATERIALS AND METHODS

Animals

Male Wistar rats (200 ± 20 g) were housed in standard laboratory conditions, under 12 h cycle (light on at 6 a.m.), in a controlled temperature (21 ± 2°C) and 70% humidity. The animals were kept in pairs in the cage (60/30/20 cm) or individually after i.c.v. cannula implantation, with water and food available *ad libitum*. Rats examined in the Vogel test were water deprived for 23 h daily during 4 days preceding the test session.

Intracerebroventricular injections

Surgery

Rats were anesthetized with ketamine (100 mg/kg, i.p.) and placed in a stereotaxic apparatus (Stoelting&Co., USA). A 10 mm long stainless steel guide cannula was implanted above right lateral ventricle, according to the atlas of the rat brain (3.2 mm posteriorly to the bregma, 1.5 mm

laterally to the sagital suture, 2.2 mm below the dura) (11, 12). The guide cannula was fixed to the skull with jewellery screws and dental acrylic cement. Seven days later the rats were subjected to behavioral testing.

Microinjection

Microinjections were given unilaterally using a Hamilton microsyringe connected *via* polyethylene tubings with an injection needle. The injection needle was inserted 2.0 mm below the tip of the guide cannula. S-AMPA was dissolved in 0.9% NaCl immediately before administration and injected in a volume of 5 µl at the rate of 1 µl/10 s. The injection needle was removed after 30 s and the stylet replaced. Control rats received appropriate volume of 0.9% NaCl. The behavioral tests were started 5 min after the drug administration. First, the operated animals were tested in the open field test, and after 11 days in the Vogel test.

Open-field test

Open-field testing was performed in a soundproof chamber under dim light and white noise (65 dB), without previous habituation. The apparatus consisted of two round arenas (80 cm diameter), each equipped symmetrically with 3 photocells. General activity (number of photobeam interruptions) was scored for 10 min. Simultaneously, the rats were observed by closed-circuit television and two additional parameters were measured:

— the number of entries into the central part of the open-field (this parameter was defined as a movement of an animal from the wall to the central area, over a distance of approx 15 cm).

— the time (in seconds) spent in the central sector (area defined as a centrally situated 35 cm in diameter circle).

Vogel's conflict test

Apparatus consisted of four glass boxes (30/30/25 cm), with a grid floor made of stainless steel bars. A water drinking tube was mounted on the wall of a cage. An electric shock generator was connected with the grid floor and the metal end-piece of the drinking tube. The rats were subjected to 4-day long training. During the first 2 days the animals were deprived of water 23 h daily in the home cages. During the following 2 days the subjects were placed in the experimental cages for 15 min without delivery of electric shocks. Subsequently, the rats were allowed to drink water in their home cages for 45 min. After training, the drinking of water for all animals was usually stabilized. On the fifth day, the animals were divided into two control groups receiving a solvent and experimental groups receiving appropriate drug solution. The rats were placed in the apparatus and the electric impulses were delivered every 5 sec for 4 sec long periods of time. Shock current was set at 0.4 mA. The volume of concumed water during 15 min of electric shock punishment was recorded and taken as a measure of conflict behavior.

Drugs

Diazepam (Polfa, Poland), CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) (RBI, USA), NBQX (2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo-quinoxaline) (Merz+Co., Germany), DNQX (6,7-dinitroquinoxaline-2,3-dione) (Tocris Neuramin, England), GYKI 52466 (1-(4-aminophenyl)-4-methyl-7,8-methylene-dioxy-5H-2,3-benzodiazepine) (Merz+Co., Germany) were suspended in 1% Tween 80. S-AMPA ((S)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) (Tocris Neuramin, England) was dissolved in 0.9% NaCl. Drug solutions were prepared

immediately before administration (i.p., 2 ml/kg). CNQX, NBQX, DNQX were administered 30 min before the tests and GYKI 52466 was given 60 min before the tests. Control rats received appropriate volumes of a vehicle at the same time points.

Histological analysis

After the experiment the implanted rats were sacrified. The brains were removed and stored in 5% formaldehyde solution. The frozen tissue was dissected into the slices to establish the place of microinjection. Only data from rats with the injection site located in the lateral ventricle were taken into consideration.

Statistical analysis

The data are shown as mean $\pm$ S.E.M., or in relative values as a proportion of appropriate control group results (as a percentage). The data were checked statistically using the one-way ANOVA followed by post hoc Newman-Keuls test.

RESULTS

Open field data.

Diazepam at the dose of 0.05 mg/kg significantly increased the number of entries into the central part of the open field, without changing rats motor activity. The drug prolonged also, but in not significant way, the time that animals remained in the central sector of the area (*Table 1*).

CNQX inhibited the rat exploratory behavior at the dose of 1 and 3 mg/kg i.p. (*Table 1*). The drug at the dose of 1 mg/kg significantly decreased the time spent by rats in the central sector. In these subjects appeared also a tendency to inhibit locomotor activity.

NBQX (0.1, 1 and 5 mg/kg) and DNQX (0.1, 1, 10 mg/kg) did not change the rat behavior in the open field (*Table 1*).

GYKI 52466 at the doses of 1, 10 and 20 mg/kg reduced the time the rats remained in the central sector of the open field (*Table 1*). The drug administered at the doses of 1 and 20 mg/kg decreased also the number of entries into the central part of the open field, without changing in a significant way the locomotor activity.

AMPA applied i.c.v. (0.5, 1 and 2 μg/5 μl) did not change rat behavior in the open field (*Table 1*). AMPA at the dose of 1 μg produced in 3 of 7 rats piloerection, exopthalmus, muscle tremor, stretch of the body and the tail, without loss of consciousness or clonic spasms. At the highest dose of 2 μg, AMPA evoked tonic/clonic convulsions in 1 rat (not systematized data).

Table 1. The effect of diazepam, CNQX, NBQX, DNQX, GYKI 52466 and AMPA on the behavior of rats in the open field. The data are shown as mean ± S.E.M. * $P < 0.05$ and ** $P < 0.01$ vs vehicle-treated.

Drug	Dose	n	Open field test		
			Motor activity	Central entries	Stay time in the central sector
	i.p., mg/kg				
Vevihle	—	8	114.0±41.3	9.5±4.2*	4.2±3.7
Diazepam	0.05	8	143.4±31.3	16.6±5.1*	11.6±4.0
	0.1	8	98.1±38.7	10.5±6.0	10.9±10.9
Vehicle	—	8	83.0±35.9	6.8±5.5	10.2±8.8
CNQX	0.1	8	86.4±31.7	5.4±3.1	4.7±4.3
	1.0	7	52.8±38.8	2.8±2.5	2.9±3.5*
	3.0	8	58.6±18.9	1.9±1.6*	2.3±2.5*
Vehicle	—	8	99.0±9.6	7.6±1.7	8.6±2.9
NBQX	0.1	8	82.1±12.1	5.6±1.1	5.3±2.2
	1.0	8	96.6±15.5	7.3±1.5	8.9±2.3
	5.0	8	92.3±15.5	6.9±1.8	7.3±2.7
Vehicle	—	8	78.7±8.5	5.1±1.1	5.3±1.3
DNQX	0.1	8	91.3±11.3	7.6±2.4	13.5±5.2
	1.0	8	72.4±11.6	3.0±0.9	4.3±1.6
	10.0	8	96.1±14.3	10.9±2.7	10.9±2.7
Vehicle	—	8	98.2±13.6	9.4±5.2	26.1±21.8
GYKI52466	1.0	8	69.4±38.9	3.9±3.5*	7.3±10.4**
	10.0	8	101.0±35.3	5.8±3.1	9.4±5.4**
	20.0	8	73.1±19.5	2.0±2.1**	1.0±1.9**
	i.c.v., μg/5 μl				
Vehicle	—	6	83.3±22.8	2.6±2.6	7.6±13.1
AMPA	0.5	7	71.6±51.7	4.6±7.0	10.8±22.5
	1.0	7	65.6±59.3	4.4±6.7	7.1±11.3
Vehicle	—	8	123.5±51.6	6.0±4.2	9.2±6.5
AMPA	2.0	7	63.7±61.9	2.1±2.9	3.4±6.0

Vogel's conflict test

Diazepam at the doses of 1.5 and 2.5 mg/kg significantly increased the punished consumption of water (*Fig. 1*).

CNOX did not produce any anti-conflict-like effect in the Vogel test after administration in a dose range from 0.05 to 5 mg/kg (*Fig. 1*). NBQX at the doses of 0.1, 1, 2 and 5 mg/kg did not change the rat behavior in the Vogel test (*Fig. 1*). DNQX did not produce any effect in this test after administration at the dose of 1 and 5 mg/kg (*Fig. 1*). GYKI 52466 at the doses of 0.1, 1 and 5 mg/kg did not affect the rat behavior in the Vogel test either (*Fig. 1*).

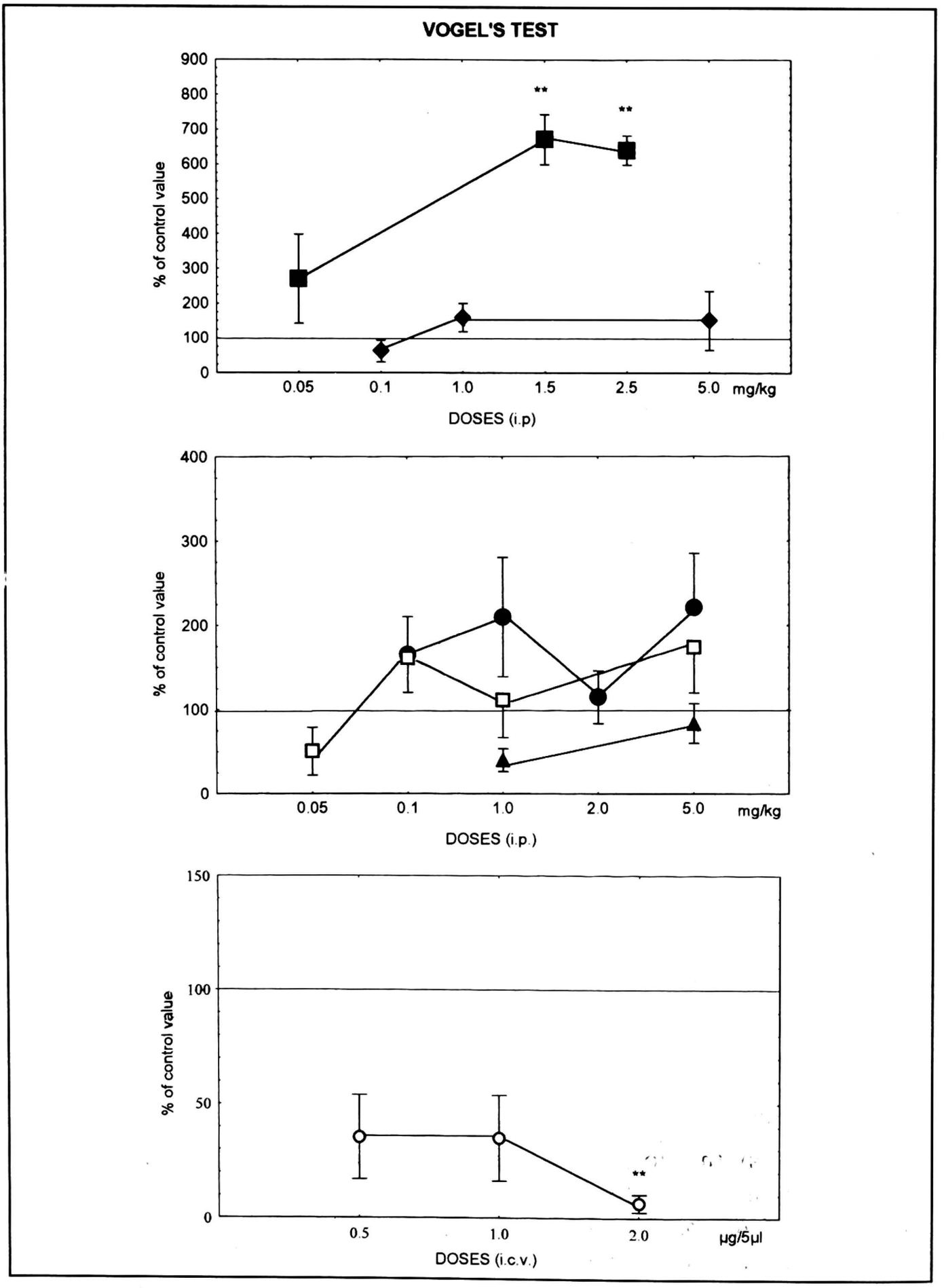

Fig. 1. The effect of diazepam, GYKI 52466, CNQX, NBQX, DNQX and AMPA in the Vogel test. The data are shown in relative values, as percentage of control group results ± S.E.M. The number of rats in the groups varied from 6—8. ■ — diazepam; ♦ — GYKI 52466, □ — CNQX, ● — NBQX, ▲ — DNQX; ○ — AMPA. * — differs from control. ** = $p < 0.05$.

AMPA administered centrally at the doses of 0.5 and 1 μg/5 μl had no influence on punished drinking (*Fig. 1*). However, when the drug was given at the dose of 2 μg, it significantly enhanced the suppressive effect of shock on drinking.

DISCUSSION

The present study demonstrated that the classic benzodiazepine receptor agonist, diazepam, significantly increased punished consumption of water in the Vogel test, and disinhibited rats reaction to a novel environment in the open field test. These effects of the benzodiazepine are known to be independent of changes in rat motor activity, spontaneous water intake and pain treshold (12, 13). The open field test appeared to be very sensitive to the benzodiazepines, as both diazepam at the very low dose of 50 μg/kg and chlordiazepoxide administered in a similar dose range, produced anxiolytic effect in this test (13, 14).

Recently, there has been an explosion of interest into the possible therapeutic use of antagonists at the AMPA/kainate receptors (7, 10, 15). Theoretically, inhibition of the excitatory amino acid receptors could be considered as alternative strategy for the treatment of several disorders of the central nervous system, such as ischemia, epilepsy and anxiety. Accordingly, NMDA receptors were shown to participate, among others, in the control of central emotional processes (9). To further clarify the role of excitatory amino acids in the control of emotional behavior, the present study was designed to evaluate the effects of AMPA/kainate receptors in two animal models of differently evoked anxiety states. In order to avoid nonspecific changes in animals gross behavior the doses of the drugs used in the experiment were lower than those shown previously to posses anticovulsant and antiischemic potential (16, 17).

The results obtained in the open field suggest inhibition of rat exploratory behavior by both competitive and noncompetitive AMPA/kainate receptors antagonists. This anxiogenic-like effect seems however to be false-positive, as in almost all instances animal motor activity tended to be depressed as well. Other evidence suggested that AMPA/kainate receptor antagonists do not appear to be effective in rodent models of learning and memory in which benzodiazepines have effect, and that they decrease the motor activity (18—21). Accordingly, CNQX (1, 3 and 10 mg/kg) was found to inhibit in a dose-dependent way the locomotor activity of rats and mice (19). NBQX caused also inhibition of the motor activity at the doses of 5, 10 and 30 mg/kg (19). Similarly, GYKI 52466 attenuated rats motility at the dose of 10 mg/kg (20). It was also found, that after administration of these drugs at the doses of

10 and 30 mg/kg, rats showed lower levels of vertical and horizontal activity in the automated open field (21). It should be stressed that some authors observed selective anxiolytic-like action of intra-amygdala infusions of CNQX in elevated plus maze (22). This effect was comparable to that produced by benzodiazepine (22). This finding is not supported, however, by many others indicating a primary role of AMPA/kainate antagonists in the motor activity regulation (see above). The injection of NBQX into the nucleus accumbens had no effect on rats locomotor activity (23), but ambulation brought about by intraaccumbens AMPA was effectively inhibited by NBQX (23). DNQX applied into the nucleus accumbens and the ventral pallidum also did not change rats motor activity (24). However, this drug reduced locomotor stimulation produced by d-amphetamine (24). Apparently, the AMPA/kainate receptors affect in a complex way rodents locomotor activity, as there is evidence that intra-amygala injections of CNQX increased rats locomotor activity (22). The infusions of CNQX also blocked the decrement of locomotion production by previous exposure to a footshock (22). It is noteworthy, that competitive and non-competitive antagonists at the AMPA/kainate receptors exerted potent anticonvulsive and neuroprotective effects at the doses higher than 30 mg/kg (10). Moreover, after the doses higher than 60 mg/kg these drugs were shown to produce potent central depressant effects, as evidenced by impairment of rotarod performance and altered EMG (17, 25). There are some data indicating that these general inhibitory properties of AMPA/kainate receptor antagonists may be of metabolic origin. For instance, it was found that CNQX potently decreased glucose utilization in the limbic forebrain (26). All in all, the open field results point at the lack of specific effects of antagonists at the AMPA/kainate receptors on rat neophobic-like reaction.

In the Vogel conflict test, among all examined AMPA/kainate receptor ligands only S-AMPA significantly altered punished consumption of water. However, its anxiogenic-like effect was accompanied by symptoms of prodromal epileptic-like activity. After the same dose of S-AMPA, some rats expressed also a clear-cut tendency to inhibit motor activity. Thus, the effect of S-AMPA seems to be not selective, and secondary to changes in rat gross behavior. Nevertheless, the possibility that S-AMPA and antagonists at the AMPA/kainate receptors may be more directly involved in the control of emotional behavior is still open as some of these drugs were found to increase anxiogenic-like behavior of rats in the plus-maze test in a dose-dependent way (27). NBQX at the dose of 10 mg/kg reduced the time spent by mice in open arms of the plus-maze test, without changing their motor activity (27). The paucity of data and disparate findings (compare 22, 27) do not allow however, to draw any univocal conclusion in this respect.

The results of this study showed that AMPA/kainate receptor antagonists, CNQX and GYKI 52466 exhibited generally inhibitory influence on

organization of rat behavior. In contrary to the NMDA antagonists, AMPA/kainate receptor antagonists appeared to not produce anxiolytic effects in both examined animal models of anxiety (9). However, the lack of selective ligands at the AMPA and kainate receptors makes the separate characterization of these receptors, and their roles in different behaviors, difficult. Thus, the availability of more precise information about the functioning of AMPA/kainate receptors is hampered by the lack of selective receptor agonists and antagonists, as well as by problems with their solubility and pharmacokinetics. Finally, it seems that AMPA/kainate receptor antagonists may be more suitable as neuroprotective and anticonvulsant agents, and as such these substances can be still considered interesting in the therapy of some neurological disorders.

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Author's address: A. Płaźnik, Department of Experimental and Clinical Pharmacology, Medical Academy, Krakowskie Przedmieście 26/28, 00-927 Warsaw, Poland.