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A Slight Anticonvulsant Effect of CNQX and DNQX as Measured by Homocysteine- and Quisqualate-Induced Seizures

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JURSON, P. A. AND W. J. FREED. *A slight anticonvulsant effect of CNQX and DNQX as measured by homocysteine- and quisqualate-induced seizures*. PHARMACOL BIOCHEM BEHAV **36**(1) 177–181, 1990.—CNQX and DNQX are compounds that have recently been reported to show potent non-NMDA excitatory amino acid receptor antagonist activity. Effects of these compounds on seizures induced by homocysteine thiolactone and quisqualic acid were studied in order to examine the pharmacological properties of these compounds. In a dosage of 1.16 μg intracerebroventricularly (ICV), CNQX prolonged the latency to the onset of quisqualate-, but not homocysteine-induced seizures. DNQX was not effective when given either ICV or systemically, although a 3.78 μg dose of DNQX given ICV markedly increased the variability in latency to seizure onset, suggesting a combination of pro- and anticonvulsant effects. Higher dosages of both CNQX and DNQX induced seizure-like activity after ICV injection. These data confirm that CNQX has pharmacological effects corresponding to its effects on cellular responses to quisqualate and kainate agonists, but these effects are weak and may limit its usefulness as a pharmacological tool.

CNQX DNQX Homocysteine Quisqualic acid Excitatory amino acids Glutamate Seizures Convulsions Epilepsy

THERE has been considerable recent interest in the possible role of excitatory amino acids in several neurological diseases including epilepsy, Huntington's disease, Alzheimer's disease, and cerebral hypoxia/ischemia and hypoglycemia (4, 15, 17, 18, 22). The ubiquitous excitatory amino acid glutamate and other endogenous amino acids have been implicated in epilepsy and neurodegenerative diseases because of their central excitatory and 'excitotoxic' effects (4, 15, 17, 18). These effects are mediated by the postsynaptic receptors for these amino acids. Three putative receptor subtypes have been described based upon their selectivity for specific agonists, as follows: 1) the AA1 or NMDA receptor which is stimulated by N-methyl-D-aspartate, 2) the AA2 or quisqualate receptor which is stimulated preferentially by quisqualic acid, and finally 3) the AA3 or kainate receptor which is stimulated by kainic acid (6,21). Antagonists of these receptor sites have been used to investigate the participation of these receptors in various forms of seizures. Several antagonists of the AA1 receptor are available, and have been used to study the role of this receptor type in seizure expression (4). Studies of the role of AA2 and AA3 receptors in seizures (7, 8, 19) have, however, been limited by the lack of potent antagonists. With the recent synthesis of two novel and potent non-NMDA receptor antagonists (12), 6,7-dinitroquinoxaline-2,3-dione (DNQX, FG 9041) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, FG 9065), further investigation of the role of AA2 and AA3 receptor subtypes in seizure activity may be possible.

Initial studies showed that CNQX and DNQX preferentially inhibit the quisqualate receptor subtype. IC_{50} values of 300 nM and 500 nM were obtained with [^{3}H]AMPA binding for CNQX and DNQX, respectively. These affinities for inhibition of [^{3}H]AMPA binding by CNQX and DNQX are comparable to that for L-glutamate. Both compounds were approximately one-fifth as effective at the AA3 receptor, with IC_{50} values of 1.5 and 2.0 μM, respectively, using [^{3}H]kainate binding (12). Honoré *et al.* (12) also showed that CNQX and DNQX selectively blocked the excitatory action of quisqualate and kainate on spinal neurons, while having limited effectiveness against L-glutamate or NMDA. Other studies found CNQX and DNQX to be capable of antagonizing responses to NMDA in rat cortical neurons and spinal cord, probably via the allosteric glycine site (1, 11, 13). DNQX and CNQX also block quisqualic acid- and kainic acid-induced [^{3}H]GABA release from cortical neurons, Na^+ efflux from striatal slices (5), and neural activation in hippocampal slices (2,16). Therefore, it appears that CNQX and DNQX are effective antagonists of the quisqualic and kainic acid receptor binding sites, but have additional effects on other sites, particularly including the

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glycine-mediated facilitation of NMDA receptors (1, 12, 13).

In view of neurophysiological evidence for an effect of CNQX and DNQX at the cellular level, the purpose of the current study was to investigate the actions of these compounds on the pharmacological level. The AA2 antagonist glutamic acid diethyl ester (GDEE) (9, 14, 20, 21) inhibits seizures and neuronal excitations induced by homocysteine thiolactone (7,23). Quisqualate-induced seizures can also be blocked by GDEE (19). Therefore, in the present study, seizures induced by homocysteine thiolactone (10) and quisqualic acid (19) were used as animal models to search for pharmacological effects of CNQX and DNQX.

METHOD

Animals

Adult female Swiss-Webster mice weighing 17–32 g were housed in groups of nine. Animals had free access to food and water at all times prior to experimentation. A total of 394 mice were tested, including those used for preliminary testing of dosages and other parameters.

Suspensions and Solutions

6,7-Dinitroquinoxaline-2,3-dione (DNQX, FG 9041), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, FG 9065), and quisqualic acid were obtained from Cambridge Research Biochemicals Inc. D,L-Homocysteine thiolactone HCl (HTL) was obtained from the Sigma Chemical Company. CNQX and DNQX were suspended in hydroxypropyl-beta-cyclodextrin (Molecusol; Research Biochemicals Inc.) for intracerebroventricular (ICV) injections. Isotonic saline was used to dissolve HTL and quisqualic acid and to suspend DNQX for IP and SC injections. Quisqualic acid was administered ICV freehand in a dosage of 3 μl and a 5 μl volume (3), and HTL was injected IP. All solutions and suspensions were made fresh daily, wrapped in foil and kept on ice during use.

Procedure

For each testing session six to twelve mice were placed individually into clear plastic cylinders 16 cm in diameter and 23 cm high. The animals were allowed to acclimate to the cylinders for ten min before testing.

Experiment 1. Effects of ICV CNQX on HTL-induced seizures. Each animal received a 5 μl ICV injection of CNQX (3.48 μg, 1.16 μg, or 0.348 μg/5 μl) or 5 μl of vehicle. Ten min following pretreatment, 825 mg/kg HTL was injected IP in a volume of 10 mg/kg.

Experiment 2. Effects of ICV CNQX on quisqualate-induced seizures. Each mouse received a 5 μl ICV injection of CNQX (3.48 μg, 1.16 μg, or 0.348 μg/5 μl) or vehicle. After 10 minutes, 5 μl of quisqualic acid solution (3 μg/5 μl) was administered ICV. Both drugs were administered on the right side of the brain.

Experiment 3. Verification of Experiment 2. Each animal received a 5 μl injection of either CNQX (1.16 μg/5 μl) or vehicle. After 20 min, each mouse received 5 μl of quisqualic acid (3 μg/5 μl).

Experiment 4. Time course of ICV CNQX on quisqualate-induced seizures. Each mouse received a 5 μl ICV injection of CNQX at 1.16 μg/5 μl. Five μl of quisqualic acid (3 μg/5 μl) was administered 5, 10, 20, or 40 min after CNQX.

Experiment 5. Effect of ICV DNQX on quisqualate-induced seizures. DNQX (12.6 μg/5 μl, 3.78 μg/5 μl, or 1.26 μg/5 μl) or vehicle (5 μl) were administered to each animal ICV. After ten min, quisqualic acid (3 μg/5 μl) was administered.

Experiment 6. Effect of IP DNQX on homocysteine-induced seizures. Mice received DNQX (126 mg/kg, 37.8 mg/kg, or 12.6 mg/kg) or vehicle IP. After 30 min each animal received homocysteine (750 mg/kg IP).

Experiment 7. Effects of IP DNQX on quisqualate-induced seizures. Mice received DNQX (126 mg/kg, 37.8 mg/kg, or 12.6 mg/kg) or vehicle IP. After 30 min animals received quisqualic acid (3 μg/5 μl ICV).

Experiment 8. Effect of SC DNQX on quisqualate-induced seizures. Each mouse received an SC injection of DNQX (126 mg/kg, 37.8 mg/kg, or 12.6 mg/kg) or vehicle SC. After 30 min animals received quisqualic acid (3 μg/5 μl).

Experiment 9. Time course of ICV DNQX on quisqualate-induced seizures. The dose of 3.78 μg DNQX was chosen because it showed the greatest tendency to inhibit quisqualate-induced seizures in Experiment 5. Each mouse received DNQX (3.78 μg/5 μl) or vehicle (5 μl) ICV. Animals received quisqualic acid (3 μg/5 μl) ICV after 30 sec, 10 min, 20 min, or 40 min.

Postinjection Observation Period

Animals were observed for 90 min after the second injection. Latency (time from the second injection to onset of the first seizure) and duration (time from seizure onset until the end of the final seizure or end of the ninety-min period, whichever came first) of any seizure activity were recorded. Animals were euthanized by CO_2 inhalation immediately at the conclusion of the ninety-min observation period.

Data were analyzed nonparametrically by the Mann-Whitney or Kruskal-Wallis H-tests, and are presented as medians ± semi-interquartile ranges. All probabilities are two-tailed.

RESULTS

Description of Seizures

For HTL-induced seizures the time of onset of the first motor seizure (running fit) was scored as the time of seizure onset. Subsequently, the animals usually alternated nonsystematically between motor fits, clonic seizures, tonic seizures, or incapacitation. During incapacitation, the animals did not react to stimuli. The end of the seizures was taken to be the point at which the animals began to move about in a normal manner or respond to a tactile stimulus.

Seizures induced by quisqualic acid differed markedly from those induced by HTL. Seizures induced by quisqualic acid generally began with a state of incapacitation or more frequently a state of "rearing seizure." "Rearing seizures" were defined by the following characteristics: 1) Protracted rearing on the hind legs with the front paws held in front of the trunk and under the head. 2) Tail rigid and held off of the ground. 3) Eyelids closed or nearly closed. 4) Short periods (i.e., 5 seconds) of grooming or walking sometime interrupt, but the rigid position quickly returns. 5) The end of these "rearing" seizures was indicated by resumption of normal behavior and a positive reaction test, or by progression to another stage of seizure. Another unusual form of seizure induced by quisqualic acid was the "rotational" seizure. "Rotational" seizures were characterized by rapidly alternating rearing and walking around the cylinder in one direction. Additionally, motor fits were seen in some of the animals. Death sometimes occurred following tonic-ophistotonic seizures. As with the HTL seizures, the end of the seizures was taken to be the appearance of normal activity and a positive reaction to stimuli.

Surprisingly, CNQX and DNQX at higher dosages also induced seizure-like phenomena. These were distinct from the

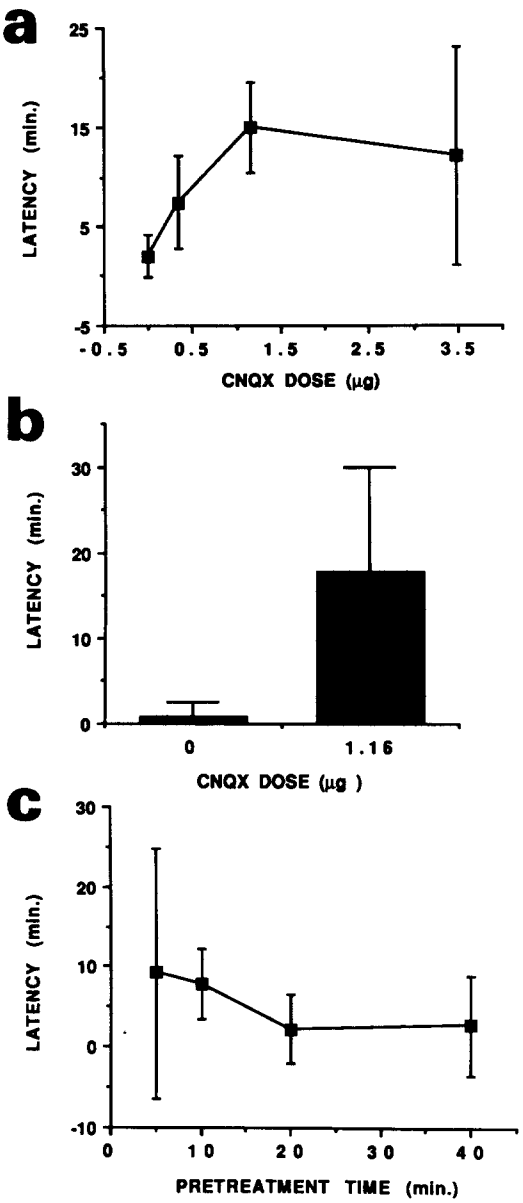

FIG. 1. Effects of CNQX on seizures induced by quisqualic acid. a) Latency to seizure onset following ICV injections of quisqualate as a function of CNQX dosage in μg (pretreatment time = 10 min). The effect of dosage was statistically significant ($H = 10.30$, $p<0.025$). $N = 8$ for all groups except $n = 10$ for the vehicle group. b) Replication of the effect shown in "a." Latency to the onset of seizures after injection of quisqualate is shown for animals pretreated with 1.16 μg CNQX or vehicle. $N = 5$ per group. c) Latency to seizure onset following ICV administration of quisqualate as a function of pretreatment time. $N = 8$ per group. Error bars represent S.I.Q.R.

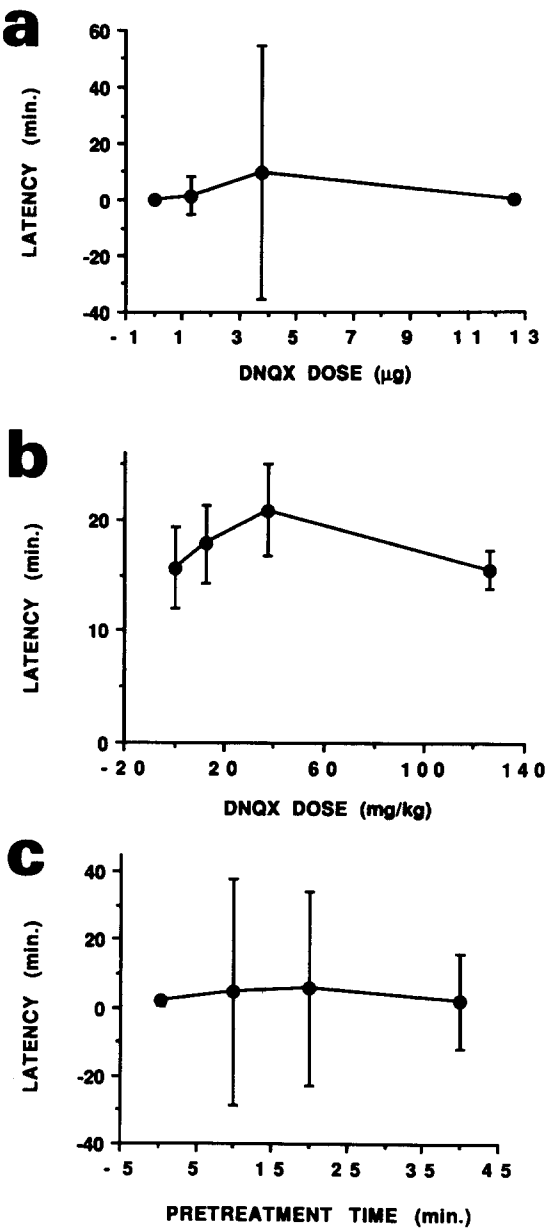

FIG. 2. Effects of DNQX on seizures induced by quisqualic acid and HTL. a) Latency to seizure onset following ICV administration of quisqualate as a function of DNQX (ICV) dosage (pretreatment time = 10 min). $N = 8$ for all groups except $n = 7$ for the largest dosage. b) Latency to seizure onset following administration of HTL as a function of DNQX (IP) dosage. $N = 8$ per group. c) Latency to seizure onset following administration of quisqualate, as a function of time between DNQX (ICV) and quisqualate (ICV) injection. $N = 8$ per group. Error bars represent S.I.Q.R.

seizures produced by HTL or quisqualic acid. Approximately one minute after receiving ICV injections of CNQX (11.6 and 3.48 μg) or DNQX (12.6 and 3.78 μg), the animals developed "scratching" seizures. These seizures were characterized by repetitive stereotyped low amplitude grooming movements with the hind legs. At the higher dosages this was more pronounced and included falling from side to side. Onset was sudden, and sometimes preceded by a state of incapacitation. These "scratching" episodes varied in length, but generally were most intense approximately 5 min following the initiation of the seizure. These "scratching" seizures were distinct from grooming stereotypy in the rapidity and intensity of the movements, and thus were classified as seizures. Seizures were not observed in any of the animals that received DNQX via IP or SC injection. Animals that received DNQX via IP or SC injection produced urine that was distinctly orange in color, suggesting the presence of DNQX in the urine within a few min after injection.

Effects of CNQX

CNQX, when administered via ICV injection, was ineffective at blocking or reducing the latency ($H = 1.786$, $p > 0.05$) and duration ($H = 1.145$, $p > 0.05$) of seizures induced by HTL.

The effect of CNQX (ICV) on quisqualate-induced seizures was significant in terms of latency ($H = 10.305$, $p < 0.025$) and duration ($H = 10.002$, $p < 0.025$). Figure 1a shows a dose-response curve for the effect of CNQX on the latency to seizure onset. Note that the effect was diminished at the highest (3.48 μg) dose. The ability of the 1.16 μg dose to effectively inhibit the seizures was verified in a second experiment (Fig. 1b). The decrease in seizure duration was secondary to the increased latency, in that most of the animals that began to seize at any point during the observation period either died or continued to have seizures for the duration of the observation period.

Figure 1c represents the time course for the effect of 1.16 μg of CNQX. There was no difference between the various times in terms of latency ($H = 2.281$, $p > 0.25$) or duration ($H = 3.259$, $p > 0.25$). The greater variability that was seen for the shorter pretreatment times may indicate an undefined short-term effect of the drug.

Effects of DNQX

DNQX did not block quisqualate-induced seizures. DNQX was ineffective at causing a significant change in latency ($H = 7.381$, $p > 0.05$; Fig. 2a) or seizure duration ($H = 2.986$, $p > 0.25$). It is interesting to note that the 3.78 μg dose produced a much greater variability than the other doses tested. This large variability was due to the fact that nearly half of the animals seized almost immediately while the other half of the animals did not seize at all.

DNQX did not inhibit HTL-induced seizures when injected IP. A tendency of higher doses to increase latency (Fig. 2b) was not significant ($H = 4.17$, $p > 0.1$), and disappeared at the highest (126 mg/kg) dosage. There was also no effect of IP DNQX on seizure duration.

Systemic DNQX also did not inhibit quisqualate (ICV)-induced seizures. The effects of DNQX on latency following IP ($H = 7.525$, $p > 0.05$) or SC ($H = 0.077$, $p > 0.25$) pretreatment were not significant. Differences in duration following IP ($H = 1.554$, $p > 0.25$) and SC ($H = 1.179$, $p > 0.25$) pretreatment were also not significant (data not shown).

For ICV-injected DNQX, there was no effect of pretreatment time on seizures induced by quisqualate. Effects on latency ($H = 1.228$, $p > 0.25$) and duration ($H = 0.317$, $p > 0.25$) were not significant. Figure 2c shows that the variability in the latency to seizure onset was markedly increased for short (10–20 min) pretreatment times, reminiscent of the effect that was seen in the time couse of ICV CNQX vs. quisqualate (Fig. 1c).

DISCUSSION

Two new compounds, CNQX and DNQX, have AA2 and AA3 antagonist properties (12), although very little data on their pharmacology is available. In the present study, pharmacological evidence that CNQX is capable of inhibiting seizures induced by quisqualic acid was obtained. This agrees with binding studies suggesting that CNQX acts via the AA2 receptor and is capable of antagonizing the excitatory actions of quisqualate and kainate (12). Effects of CNQX were, however, weak and seen over a very narrow dosage range, and neither compound inhibited HTL-induced seizures. Also, some dosages of CNQX and DNQX that did not produce overall changes caused a marked variability in the time to seizure onset. This tends to suggest that these compounds are producing both anticonvulsant and proconvulsant effects which are differentially manifest under various circumstances.

The present data show that the properties of CNQX as a quisqualate receptor antagonist can be manifest at the pharmacological level, although these effects are weak and seen only under restricted circumstances. Moreover, both CNQX and DNQX appeared to have mixed or nonspecific properties, because of their apparent tendency to promote seizures in higher dosages. These data are not encouraging for the use of CNQX as a pharmacological tool, although it is possible that related compounds can be found that would be more effective. Failure to observe more marked effects of CNQX and DNQX in the present study may be due to pharmacokinetic and bioavailability variables, or possibly the choice of assay methods. Nevertheless, using the same seizure assays, GDEE appears to be a more effective agent than CNQX. The present study does show that CNQX can produce an anticipated pharmacological effect, inhibition of quisqualate-induced seizures. This confirms that the reported cellular effects of CNQX correspond at least in part to its effects on overt seizure activity.

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