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Influence of Kynurenine Treatment on Open-Field Activity, Elevated Plus-Maze, Avoidance Behaviors and Seizures in Rats

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VÉCSEI, L. AND M. FLINT BEAL. *Influence of kynurenine treatment on open-field activity, elevated plus-maze, avoidance behaviors and seizures in rats.* PHARMACOL BIOCHEM BEHAV **37**(1) 71–76, 1990.—In the present studies the effects of single and daily repeated injections of kynurenine were investigated in different behavioral tests in rats. In open-field behavior a single injection of kynurenine decreased rearing activity, while the effect was more pronounced after repeated injections. Similarly, in the elevated plus-maze, kynurenine attenuated the total number of entries in the four arms of the equipment, and after chronic treatment decreased the time spent in the open arms (control: 24%; kynurenine 100 mg/kg: 16%; kynurenine 200 mg/kg: 13%). Kynurenine did not influence the passive avoidance learning paradigm (learning session and avoidance latency) and the extinction of active avoidance response. Kynurenine slightly attenuated the kainic acid-induced wet dog shakes and forelimb clonic activity with rearing. These findings suggest that kynurenine (especially after repeated peripheral injections) inhibited several behavioral responses of the experimental animals. However, in one type of highly motivated experimental paradigm (fear from the foot shock), behavioral depression was not detectable.

Behavior Kynurenine Kainic acid Rats

KYNURENINE (KYN) is an intermediate compound in the metabolism of tryptophan to nicotinamide-adenine dinucleotide (NAD^+) (3). It has been identified as a constituent of rodent and human brain at an average concentration of approximately 1 μM (5). Interest in KYN has increased markedly since it was discovered that two of its metabolic products, kynurenic acid and quinolinic acid, act as an antagonist and agonist, respectively, at receptors for excitatory amino acids (20).

Kynurenic acid is an endogenous compound which exerts neuroprotective effects when coinjected with quinolinic acid (2). Quinolinic acid, a selective ligand at the N-methyl-D-aspartate (NMDA) receptor (19), has been suggested to be an important pathogenetic factor in neurodegenerative disorders, such as Huntington's disease, hypoxia, hypoglycemia and epilepsy (1,14).

Since KYN is metabolized to both excitatory (quinolinic acid) and inhibitory (kynurenic acid) compounds in the body (more detailed description of the biochemical processes are in the Discussion section), in the present studies we investigated the behavioral effects of peripherally administered KYN in different behavioral tests after both single and repeated injections. In chronic studies the animals were treated with KYN 4 consecutive days since their conditional avoidance responses markedly decreased during the extinction trials. KYN was given 2 hr before the behavioral tests since peripheral administration of KYN resulted in marked dose-dependent increases in striatal extracellular fluid concentrations of kynurenic acid, peaking at 2–2.5 hr (21).

METHOD

Animals

Male Sprague-Dawley rats (Charles River) weighing 160–200 g were housed in cages (2–4 animals/cage) with ad lib access to chow and water. A 12:12-hr light-dark cycle was maintained (light on 0600 hr), and temperature and humidity were controlled. The animals were handled for 5 min once daily during 4 consecutive days before the experiments.

Drugs and Treatments

L-Kynurenine-sulfate (KYN) and kainic acid (KA) (Sigma,

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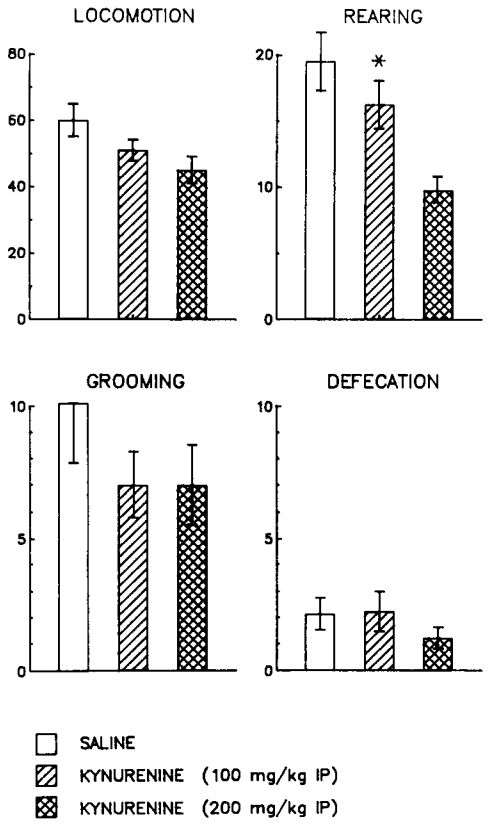

FIG. 1. Effects of a single injection of kynurenine on the open-field activity of rats. Vertical lines represent the standard errors of the mean. (n = 10 animals/group.) There were dose-dependent significant reductions in rearing (*$p<0.02$, Kruskal-Wallis ANOVA), but smaller reductions in locomotion, grooming and defecation were not significant.

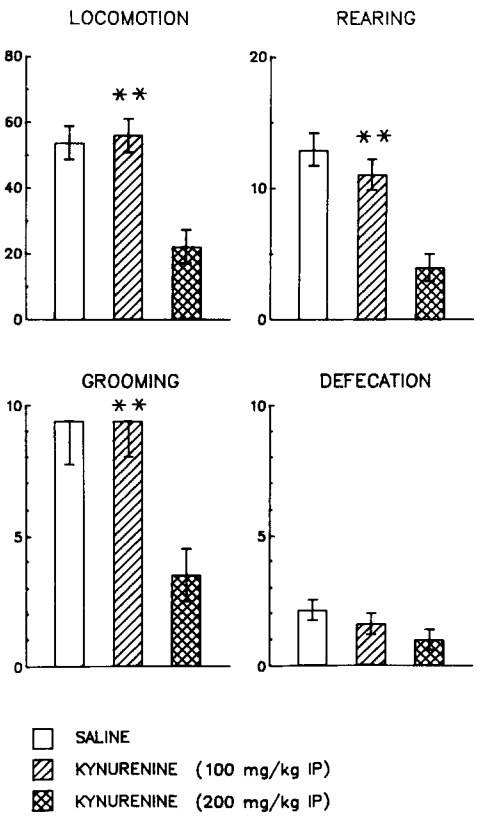

FIG. 2. Effects of repeated injections of kynurenine on the open-field activity of rats. Vertical lines represent the standard errors of the mean. (n = 10–12 animals/group.) There were dose-dependent significant reductions in locomotion, rearing and grooming (**$p<0.01$, Kruskal-Wallis ANOVA).

St. Louis, MO) were used. KYN was injected in doses of 100 or 200 mg/kg (free base) intraperitoneally (IP) once in the acute experiments and on four consecutive days in the chronic studies. The compound was administered 2 hr before the behavioral tests or KA injection.

Behavioral Tests

Exploratory activity. The animals were placed in an open-field box (100 by 100 cm, 40 cm high), white wooden box, the floor consisting of 25 equally sized squares measuring 20 by 20 cm each. The activity during a three-min session was videorecorded (VHS Movie, VX-406, Olympus, Olympus Optical Co. Ltd., Tokyo, Japan). During the experimental sessions, the testing room was illuminated with dimmed white light. Their behavior was characterized by the total number of squares (horizontal activity), the total number of rearings (vertical activity), the number of groomings and the number of defecation boluses produced during the 3-min session. The open-field test was performed 2 hr after IP injection of KYN (100 mg/kg; 200 mg/kg free base) or saline (8 ml/kg).

Elevated plus-maze test. The experimental device was an elevated (1 m above ground) plus-formed maze, which was placed in a dim room. The four arms were 40 cm long and 10 cm wide, with mesh wire floors. Two opposing arms were surrounded by black 10 cm high walls (closed arms), while the other arms were devoid of walls (open arms). The testing was immediately preceded by the open-field test. The placement of the animal in an unfamiliar environment prior to the test increases the total number of entries (12), increasing the sensitivity of the test (4). Thereafter, the rat was placed in the centre of the maze, facing a closed arm. The investigator was situated approximately 2 m from the center of the maze. Entry into an arm was defined as the animal placing all four paws in that arm. The cumulative time spent in, as well as the number of entries made into, the open or closed arms were recorded during a five-minute test session. The open-arm data were then expressed as percent of the total time spent in, and of the total number of entries made into, both closed and open arms. The plus-maze was carefully wiped with a wet towel after each tested animal.

Passive avoidance behavior. A single session step-down, passive avoidance reaction was investigated (Lafayette Instrument, Lafayette, IN). Two hr after kynurenine (100 or 200 mg/kg IP) or saline administration the animals were placed on the grid floor of a bench-jumping conditioning apparatus and were trained to jump onto the bench. Current (0.2 mA, 1 sec) was applied through the grid floor until the rats jumped onto the bench and the total number of foot-shocks in each session to reach the criterion (remaining on the bench for 180 sec) was measured. After having met this criterion, the rats were immediately removed from the

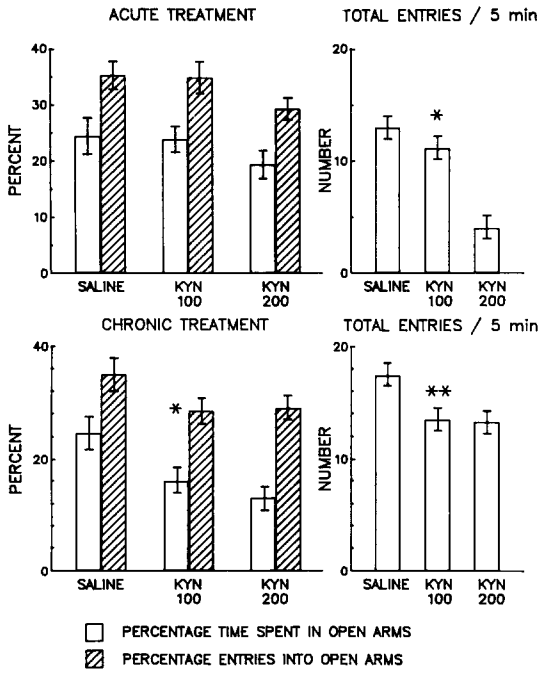

FIG. 3. Effects of single and repeated administration of kynurenine on the behavior of rats in the elevated plus-maze. *$p<0.02$, **$p<0.01$ (Kruskal-Wallis ANOVA). Vertical lines represent the standard errors of the mean. (n = 10–12 animals/group.) There were significant reductions in the time spent in the open arms in the chronically treated animals and in total entries in both the acutely and chronically treated animals.

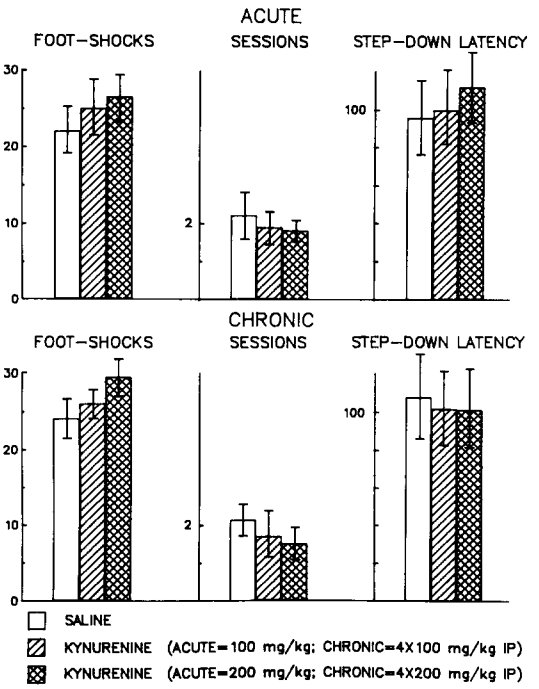

FIG. 4. Effects of single and repeated administration of kynurenine on the passive avoidance test. Vertical lines represent the standard errors of the mean. (n = 10–12 animals/group.) There were no significant effects.

apparatus and 24 hr later each animal was again placed on the bench and the latency to step-down ("step-down latency") was measured to a maximum of 180 sec (6).

Active avoidance behavior. Active avoidance conditioning was performed in a bench-jumping conditioning apparatus. The conditional stimulus (CS) was the light of a 40-W electric bulb, while the unconditional stimulus (US) was an electric shock of 0.2 mA (1 sec) delivered through the grid floor of the apparatus to the paws of the rat (23,24). Each day 10 trials were performed, with a mean intertrial interval of 60 sec. Animals which made at least 8 conditional avoidance responses out of 10 trials in the learning session were used for further experiments. These rats were allocated to two different treatment groups and injected on the next day IP 2 hr before the extinction session with KYN (200 mg/kg) or saline. When the extinction trials were run the US was no longer applied. The CS was presented for a maximum of 10 sec, or it was terminated as soon as the animal made the response.

Kainic acid-induced seizure. Two hr after acute (200 mg/kg IP) or chronic (daily 200 mg/kg IP during four consecutive days) KYN-pretreated rats were injected with KA (10 mg/kg IP). Control animals received physiological saline and KA. After the KA treatment the animals were placed immediately in individual cages and their behavior was videorecorded during a 110-min period. Behavioral changes were rated with Lassmann and co-worker's scale (8) as modified by Sztriha and co-workers (22); 0 = normal behavior; 1 = wet dog shakes (WDS) and "freezing"; 2 = WDS + weak clonic convulsions of forelimbs and head; 3 = forelimb convulsions with rearing, chewing, sniffing and hypersalivation; 4 = continuous licking, limbic motor seizures with loss of postural control, circling movements, and intense agitation. Additionally, the total number of WDS was recorded every ten minutes. The videotapes were carefully evaluated in separate sessions.

Statistics

Kruskal-Wallis analysis of variance by ranks was performed with respect to the treatment effects.

RESULTS

A single injection of KYN dose-dependently decreased the rearing ($H = 8.83$, $p<0.02$), and slightly but not significantly attenuated the locomotor, grooming and defecation activity of the animals (Fig. 1).

Daily repeated injections of KYN on the 4th day decreased the locomotor ($H = 16.74$, $p<0.01$), rearing ($H = 18.73$, $p<0.01$) and grooming ($H = 9.69$, $p<0.01$) activities of the rats as compared to the saline-treated control group (Fig. 2). Responses were dose-dependent being much more marked at 200 than at 100 mg/kg.

In the elevated plus-maze a single injection of KYN significantly decreased ($H = 7.98$, $p<0.02$) the number of total entries into the four arms of the equipment. The percentage time spent in, and the entries into, the open arms of the maze only slightly differed in the treatment groups. The chronic administration of KYN decreased the number of total entries ($H = 10.07$, $p<0.01$) and the percentage time spent in the open arms ($H = 8.44$, $p<0.02$) (Fig. 3).

KYN (after single or repeated injections) did not significantly influence the passive avoidance learning session and the avoidance latency measured 24 hr after the learning trial (Fig. 4). KYN (administered daily in a dose of 200 mg/kg IP) had no effect on the

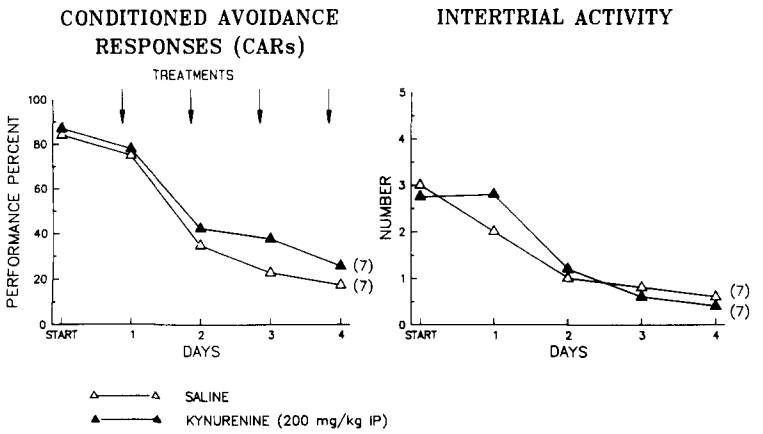

FIG. 5. Effects of repeated injections of kynurenine on the extinction of active avoidance response in rats. (n = 7 animals/group.) Kynurenine had no significant effect.

extinction and intertrial activity of the active avoidance response of the experimental animals (Fig. 5).

The behavioral alterations induced by systemic KA were similar to those described previously (8). The first behavioral changes seen after KA administration were WDS and intermittent periods of immobility (freezing). The frequency of WDS gradually decreased as the behavioral seizures (facial muscle myoclonus, forelimb clonus, rearing, hypersalivation) appeared. The seizures were fully developed 90–110 min after KA injection; they were characterized by constant head nodding, rearing, and occasional falling (median behavioral rating = 3). We selected, and during the videotape analysis counted the number of animals which showed the well-characterized grade 3 syndrome (forelimb convulsions with rearing). A single injection of a high dose of KYN only slightly attenuated the number of WDS, but consistently decreased the development of forelimb convulsions with rearing. The repeated injections of KYN (4 × 200 mg/kg/day IP) decreased the number of WDS at 30 min ($H = 8.04$, $p < 0.05$) and 50 min ($H = 10.06$, $p < 0.01$) after treatment with KA and delayed the development of behavioral signs of grade 3 (60 min: $H = 6.57$, $p < 0.05$; 70 min: $H = 12.01$, $p < 0.01$) (Fig. 6).

DISCUSSION

Speciale *et al.* (16) demonstrated that peripheral injection of

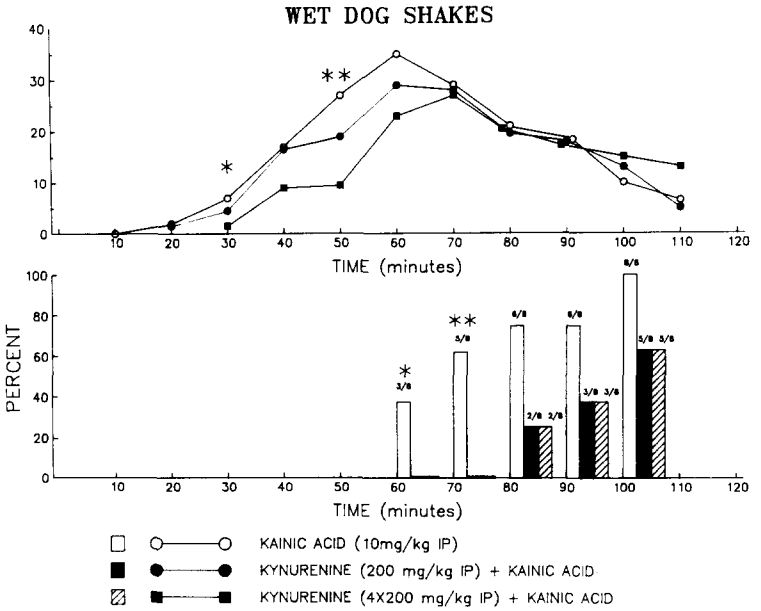

FIG. 6. Effects of kynurenine pretreatment on kainic acid-induced seizures. Columns represent the development of grade 3 (forelimb convulsions with rearing). $*p < 0.05$, $**p < 0.01$ (Kruskal-Wallis ANOVA). (n = 8 animals/group.) Repeated injections of kynurenine significantly decreased the number of wet dog shakes at 30 and 50 minutes and inhibited the development of grade 3 at 60 and 70 minutes.

KYN causes rapid and dose-dependent increases in the serum level of quinolinic acid. However, intravenous injection of large doses of quinolinic acid induced only minor electroencephalographic modifications and no neurotoxicity in rats with a mature blood-brain barrier (BBB) (25). Recent experimental findings (17) suggested that a KYN metabolite such as 3-hydroxy-KYN, or possibly another metabolite of KYN, is produced in the periphery, penetrates through the BBB and subsequently acts as a bioprecursor of locally synthesized quinolinic acid. This appears to be the case since KYN or tryptophan infusion through a dialysis probe in the striatum does not result in increased levels of quinolinic acid (17). In contrast, administration of 3-hydroxyanthranilic acid results in marked increases. This finding suggests that the metabolic pathway leading from KYN to quinolinic acid may not be present in brain. Peripheral administration of KYN, however, does lead to increased levels of quinolinic acid in the brain, suggesting that it is converted to a metabolite which then crosses the BBB and serves as precursor to quinolinic acid (15). Speciale *et al.* (18) suggested that KYN may serve as a storage form of neuroactive kynurenines in the brain, which may be selectively influenced in response to neuronal activity.

Swartz *et al.* (21) found that the KYN metabolite kynurenic acid is present in extracellular fluid within the central nervous system (CNS), and the CNS can synthesize kynurenic acid and release it into the extracellular space. Peripheral administration of KYN produced a marked increase in the striatal extracellular concentration of kynurenic acid. In contrast, peripheral administration of kynurenic acid results in large increases in plasma levels but only modest increases in striatal extracellular fluid. Current evidence suggests that increased brain kynurenic acid levels may be the predominant effect of peripheral KYN administration.

In the present behavioral experiments we found that a single injection of a high dose of KYN decreased rearing activity in the open-field test and the total number of entries in the elevated plus-maze. After chronic administration of the compound the inhibitory effects on exploratory activity were more pronounced. These results are in accordance with the decreased number of total entries in the 4 arms of the elevated plus-maze. In these models, behavior is studied in a situation of conflict between a reinforced drive (exploration of the new environment) and an aversive stimulus (unconditioned aversion that rats have for new unknown environments and elevated open places).

However, in the passive avoidance behavior paradigm, KYN has no effect on the learning session, and the avoidance latency of the rats. Similarly, KYN did not influence extinction of active avoidance behavior. It is known that delay of the active avoidance response may be due to the increased selective attention to the conditioned stimulus of the light signal, a more general state of arousal, enhanced motivation, improvement in memory function or, to an unadapted perseverance of performance, as observed experimentally after lesion of the dorsal noradrenergic bundle (10). The present findings suggest that the KYN-induced behavioral depression is not observable in one type of highly motivated (fear from the foot shock) behavioral paradigms.

Olney and co-workers (11) showed that KA is a powerful neurotoxic analogue of glutamate, which causes neuronal damage. Peripheral administration of KA results in the development of persistent seizures. The KA-induced WDS are postulated to be an initial stage of the progression of limbic seizures towards generalization (13). Furthermore, the KA-induced neurotoxicity is positively correlated with seizure severity (9).

KYN (especially after repeated injections) slightly attenuated the KA-induced behavioral seizures (WDS and grade 3). These data are in agreement partly with Lapin's (7) findings, who reported that IP or orally administered KYN antagonized strychnine-induced seizures. However, in their experiments peripherally injected quinolinic acid also had anticonvulsive effects, despite the fact that this compound has neurotoxic and convulsant effects and poorly penetrates over the BBB (25). KA, however, results in breakdown of the BBB and induces brain edema (22). It is possible that after KA injection quinolinic acid penetrates over the BBB and this process explains the relatively low efficacy of KYN in preventing KA-induced seizures.

The precise biochemical metabolism of KYN and its metabolites (the ratio of peripheral and central metabolism, the turnover of biochemical processes, penetration over the BBB and diffusion in the brain tissue of different KYN metabolites, the relationship between the levels of compounds in extracellular brain fluid and concentrations at the NMDA receptor, etc.) are under investigation in several laboratories. A marked increase in kynurenic acid concentrations has been shown, however it is as yet uncertain whether quinolinic acid concentrations show similar changes. Our present findings suggest that KYN-induced behavioral depression is not observable in fear-motivated tests. There is, however, a general depression of several other behavioral parameters, such as exploration of the open-field box or elevated plus-maze. The possible anxiogenic effects of chronic treatment might merit further investigation, particularly if these can be shown to be independent of the sedative effects.

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