ELSEVIER

Pharmacology, Biochemistry and Behavior 72 (2002) 779–786

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Amino acid and monoamine alterations in the cerebral cortex and hippocampus of mice submitted to ricinine-induced seizures

Anete C. Ferraz[a], Janete A. Anselmo-Franci[b], Sandra R. Perosa[c], Eduardo F. de Castro-Neto[c], Maria Ines Bellissimo[c], Brás H. de Oliveira[d], Esper A. Cavalheiro[c], Maria da Graça Naffah-Mazzacoratti[c], Cláudio Da Cunha[a,*]

[a]*Laboratório de Fisiologia e Farmacologia do SNC, Departamento de Fisiologia e Departamento de Farmacologia, Universidade Federal do Paraná, CP 19.031, Curitiba, PR 81.531-990, Brazil*

[b]*Fac. Odontologia Ribeirao Preto, Departamento de Morfologia Estomatologia e Fisiologia, Av Cafe S-N, Ribeirao Preto, SP BR-14040904, Brazil*

[c]*Disciplinas de Bioquímica e Neurologia Experimental, Universidade Federal de São Paulo (UNIFESP-EPM), São Paulo, SP, Brazil*

[d]*Departamento de Química, Universidade Federal do Paraná, Curitiba, PR, Brazil*

Received 12 June 2001; received in revised form 15 January 2002; accepted 28 January 2002

Abstract

The alkaloid ricinine isolated from the plant *Ricinus communis*, when administered to mice at high doses, induces clonic seizures accompanied by electroencephalographic alterations in the cerebral cortex and hippocampus. The lethal nature of ricinine-induced seizures is considered to be a good model for the study of the events that cause death during clonic seizures, particularly those related to respiratory spasms. The initial signs (pre-seizure period) were marked by exophthalmus and decreased locomotor behavior. Animals killed during the preseizure period presented an increased utilization rate (HVA/DA) of dopamine (DA), an increased concentration of noradrenaline (NA), and a decreased concentration of glutamate (Glu), glutamine (Gln), taurine (Tau), and serotonin (5-HT) in the cerebral cortex. The seizure period is characterized by the occurrence of hind limb myoclonus and respiratory spasms, which are followed by death. Alterations in the cerebral cortex concentration of these neurotransmitters persisted during the seizure period. These alterations are only partially observed in the hippocampus, mainly during the seizure period. The present results suggest that an increased release of Glu in the cerebral cortex can be implicated in the genesis of the ricinine-induced seizure and that it triggers many anticonvulsive mechanisms, like the release of Tau, DA, 5-HT, and NA.

Keywords: Ricinine; Cerebral cortex; Hippocampus; Seizures; Amino acids; Monoamines; Mice

1. Introduction

Ricinus communis is a plant that induces intoxication in cattle or rabbits in several regions of Brazil. The ingestion of leaves or fruits produces lack of equilibrium and the animals are unable to walk. They also show muscle tremors, sialorrhea, and eructation. Sudden recovery or death follows these signs. In addition, the alkaloid ricinine isolated from *R. communis* leaves and fruit pericarps induces clonic seizures and central nervous system stimulation when administered at high doses to mice (EC_{50} = 25 mg/kg) (Ferraz et al., 1999, 2000) and to other mammals (Langenerger et al., 1981; Tokarnia et al., 1979).

The electrophysiological and behavioral characteristics of ricinine-induced seizures resemble some types of human seizures, such as sleep apnea syndrome and the pediatric congenital perisylvian syndrome (Devinski et al., 1995; Gropman et al., 1997). These seizures are accompanied by respiratory spasms that are followed by animal death (Ferraz et al., 1999).

The type of alteration of the neurotransmission pathways occurring in the brain of mice submitted to ricinine-elicited seizures is unknown. Previous studies (Farah et al., 1988) have suggested that ricinine may cause a mitochondrial arrest with a cyanide-like mechanism, but this explanation was contested by a recent and more careful study by Ferraz et al. (2000). According to these authors, ricinine may act

* Corresponding author. Tel.: +55-41-361-1716; fax: +55-41-266-2042.

E-mail address: dacunha@bio.ufpr.br (C. Da Cunha).

0091-3057/02/$ – see front matter
PII: S0091-3057(02)00750-5

postsynaptically by binding to a site in the benzodiazepine/$GABA_A$ ionophore complex receptor. These authors also showed that seizures induced by ricinine could be completely blocked by diazepan, which increases the EC_{50} of the convulsant effect of ricinine. In contrast, other similar compounds, such as phenytoin, and ethosuximide were not able to block ricinine-induced seizures (Ferraz et al., 2000).

Studies of other chemically induced seizures have shown that at the presynaptic level, seizure activity is associated with a wide range of local biochemical changes, affecting the release of various brain monoamines and amino acid neurotransmitters (Jacobsson et al., 1997; Li et al., 2000; Meldrum, 1995; Olsen and Avoli, 1997; Starr, 1996). In the present investigation, we studied the cortical and hippocampal concentration of several neurotransmitters such as monoamines and amino acids before and during a ricinine-induced seizure. This study aims to clarify the following questions. (1) Can alterations in these neurotransmitters point to a possible mechanism of action of the seizures induced by ricinine? (2) Do these alterations occur sooner and more intensively in a given brain region, thus suggesting the site of the epileptic activity initiation and its spreading direction? The elucidation of these questions will have potential benefits for the understanding of some epileptic seizures sharing similar mechanisms with the ricinine-induced seizures and may lead to new therapeutic approaches.

2. Methods

2.1. Drugs and injection procedures

Ricinine was isolated from the pericarps of *R. communis* as described elsewhere (Ferraz et al., 1999), dissolved in a mixture of corn oil:water (1:1), with a drop of Tween 80 per 1 ml of water, and administered to mice subcutaneously. The drugs used for neurotransmitter quantification were dihydroxybenzylamine (DHBA; internal standard), dihydroxyphenylacetic acid (DOPAC), 5-hydroxyindoleacetic acid (5-HIAA), noradrenaline (NA), homovanillic acid (HVA), 5-hydroxytryptophan (5-HT), dopamine (DA), gamma-aminobutyric acid (GABA), glutamate (Glu), glycine (Gly), taurine (Tau), glutamine (Gln), and homoserine (HSER; internal standard). All drugs were purchased from Sigma (St. Louis, MO, USA).

2.2. Animals

The experiments were performed after approval of the experimental protocol by the Ethics Committee of the institution and all efforts were made to minimize animal suffering.

Thirty adult male Swiss mice (2.5 months) from our own breeding stock, weighing 20–30 g, were used. The animals were maintained in a temperature-controlled room (22 ± 2 °C) on a 12/12-h light/dark cycle (lights on 07:00 h) with food and water available ad libitum. The animals were maintained in Plexiglas home cages (60 × 25 × 25 cm), 10 to a cage. All experiments were conducted between 13:00 and 17:00 h.

2.3. Animal treatment and biochemical assays

Ten mice were treated with saline, 10 mice received the ricinine vehicle, and another group of 20 mice received 40 mg/kg ricinine sc. Ten ricinine-treated animals were killed during the pre-seizure period, while the remaining ricinine-treated animals were killed during the seizure period ($n = 10$). The pre-seizure period was considered to be the time during which the animals presented exophthalmus, the first pre-seizure signal (5–10 min after ricinine injection) (Ferraz et al., 2000). The period related to seizure onset was identified by the occurrence of hind limb clonus (15–30 min after ricinine administration). After decapitation, the cerebral cortex and the hippocampus were rapidly dissected on dry ice and stored at −70 °C until the time for assay. The tissues were ultrasonically homogenized in a 0.1-M solution of $HClO_4$ containing 0.02% $Na_2S_2O_2$, DHBA (146.5 ng/ml) as internal standard for the monoamine assay, and HSER (10 μg/ml) as internal standard for the amino acid assay, using 15 μl solution/mg tissue. After centrifugation at 11,000 × *g* (4 °C) for 40 min, 20 μl of the supernatant was injected into the chromatographic system.

The endogenous levels of DA, 5-HT, and their non conjugated metabolites DOPAC, HVA, and 5-HIAA were assayed by reverse-phase high-performance liquid chromatography (HPLC) coupled to electrochemical detection, as described by Cavalheiro et al. (1994). The mobile phase, used at a flow rate of 0.8 ml/min, consisted of 0.02 M phosphate/citrate buffer and 90/10 methanol (vol/vol), 0.12 mM Na_2EDTA, and 0.0556% heptane sulphonic acid as ion pair. The pH was adjusted to 2.64 with H_3PO_4 at 22 °C. A 5-μm (220 × 4.6) Spheri-5 RP-18 column from Brownlee Laboratory was used. Electrochemical detection of monoamines was performed with a Shimadzu L-ECD-6A electrochemical detector with a potential of 0.75 V. The peak area of the internal standard (DHBA) was used to quantify the sample peaks.

The endogenous levels of the amino acids were analyzed after pre-derivatization, as previously described (Cavalheiro et al., 1994), and GABA, Glu, Gly, Gln, and Tau levels were assayed using a reverse-phase HPLC system. The internal standard, HSER, was used to quantify the amino acids present in brain samples. A Lichrospher 100 RP 18.5-μm (125 × 4 mm) column and an RL-10 Axl Shimadzu fluorescence detector (EX 348 nm, EM 460 nm) were used. A linear gradient was applied using mobile phase A at a flow rate of 1 ml/min, consisting of 0.05 M sodium phosphate buffer, pH 5.5, plus 0.03% NaCl/20% methanol, and mobile phase B consisting of 0.05 M sodium phosphate buffer, pH 5.5, plus 0.03% NaCl/80% methanol. The HPLC gradient system used for amino acid analysis consisted of two pumps linked to a programmable gradient controller. All HPLC analyses were done using a Shimadzu Class-LC10 computer program.

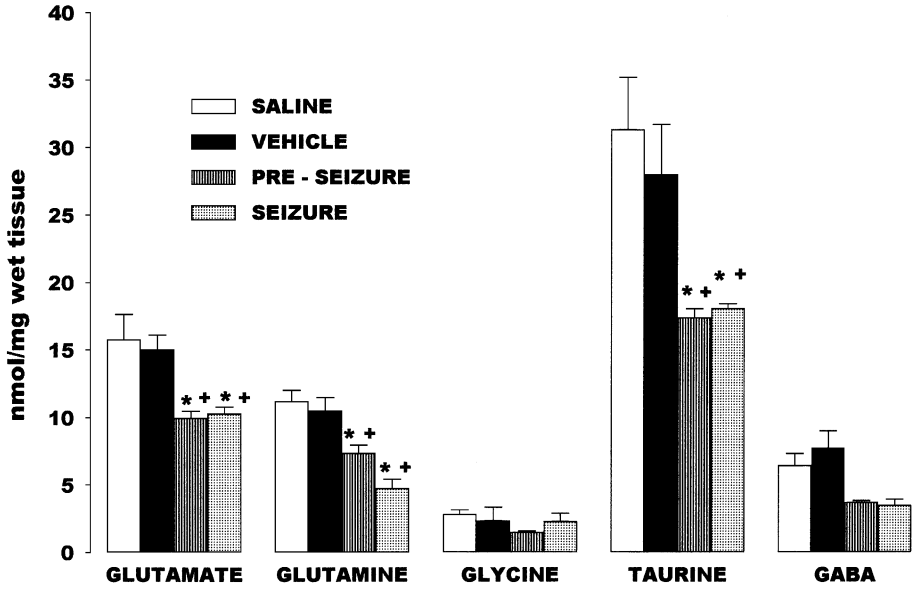

Fig. 1. Alterations in cerebral cortex amino acid concentration of ricinine-treated mice sacrificed just after the appearance of the first pre-seizure signs (5–10 min after drug administration) or during the seizure period (15–30 min after drug administration). The bars represent mean ± S.E.M. amino acid concentration. * $P \leq 0.05$ compared to the saline group; $^{+}P \leq 0.05$ compared to the vehicle group; one-way ANOVA followed by Scheffé test.

2.4. Statistical analysis

Differences between groups concerning monoamine and amino acid levels were determined by one-way ANOVA followed by the post-hoc Scheffé test.

3. Results

Four to 8 min after receiving 40 mg/kg ricinine, the mice became exophthalmic, in agreement with previous reports (Ferraz et al., 2000). Exophtalmus was the first sign, which was later followed by a seizure. A few minutes later, the animals became motionless and presented muscle tremors, jumping, dyspnea, and several myoclonic jerks evolving to a generalized tonic–clonic seizure. After this, all animals presented respiratory failure, which was followed by death no longer than 30 min after ricinine administration.

As shown in Fig. 1, in animals sacrificed during the pre-seizure period (just after becoming exophthalmic) or during the seizure period, the levels of Glu [$F(3,24) = 7.12$; $P \leq 0.05$, one-way ANOVA; $P \leq 0.05$, Scheffé test], Gln

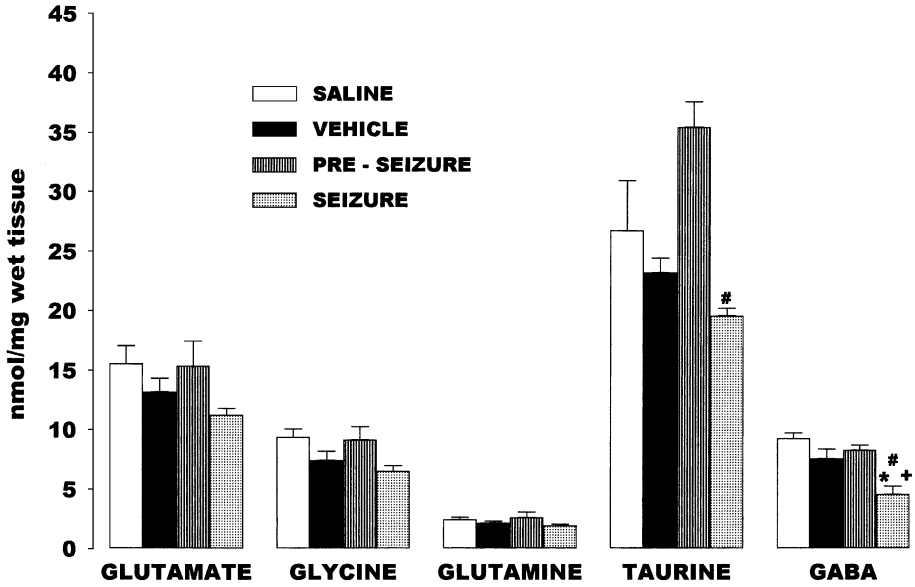

Fig. 2. Alterations in hippocampal amino acid concentration of ricinine-treated mice sacrificed just after the appearance of the first pre-seizure signs (5–10 min after drug administration) or during the seizure period (15–30 min after drug administration). The bars represent mean ± S.E.M. amino acid concentration. * $P \leq 0.05$ compared to the saline group; $^{\#}P \leq 0.05$ compared to the pre-seizure group; one-way ANOVA followed by Scheffé test.

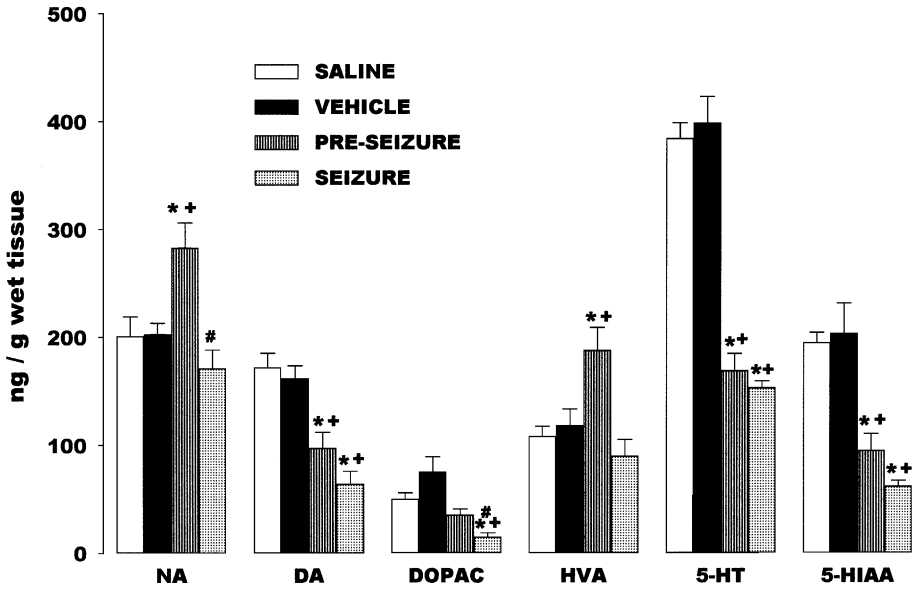

Fig. 3. Alterations in cerebral cortex monoamines in ricinine-treated mice sacrificed just after the appearance of the first pre-seizure signs (5–10 min after drug administration) or during the seizure period (15–30 min after drug administration). The bars represent mean ± S.E.M. monoamine concentrations. * $P \leq 0.05$ compared to the saline group; $^{+}P \leq 0.05$ compared to the vehicle group; $^{\#}P \leq 0.05$ compared to the pre-seizure group; one-way ANOVA followed by Scheffé test.

[$F(3,24) = 11.75$; $P \leq 0.001$, ANOVA; $P \leq 0.05$, Scheffé test], and Tau [$F(3,24) = 5.58$; $P \leq 0.05$; $P \leq 0.05$, Scheffé test] were significantly decreased in the cerebral cortex when compared to saline-treated or vehicle-treated animals. Fig. 2 shows that a significant reduction of GABA concentration occurred in the hippocampus of the animals sacrificed during the seizure period compared to the controls and to the preseizure period and a significant reduction of Tau concentration occurred compared to the pre-seizure period [$F(3,24) = 18.15$; $P \leq 0.001$; $P \leq 0.05$, Scheffé test]. Fig. 3 shows that NA [$F(3,36) = 7.10$; $P \leq 0.001$; $P \leq 0.05$, Scheffé test] and HVA [$F(3,34) = 7.50$; $P \leq 0.001$; $P \leq 0.05$, Scheffé test] concentrations were significantly increased in the cerebral cortex during the pre-seizure period; the concentrations of DA [$F(3,36) = 15.36$; $P \leq 0.001$; $P \leq 0.05$, Scheffé test], 5-HT [$F(3,36) = 64.02$;

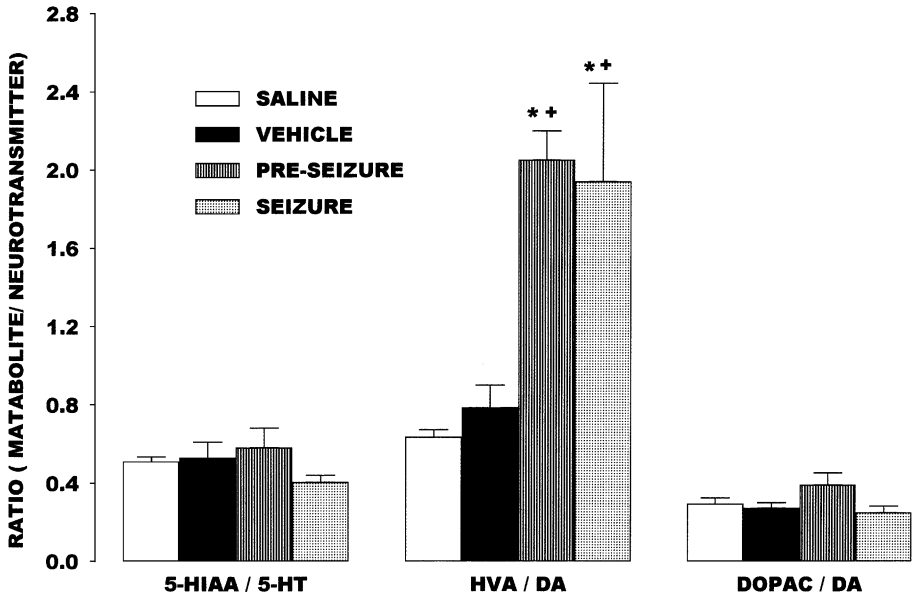

Fig. 4. Utilization rate of cerebral cortex monoamines in ricinine-treated mice sacrificed just after the appearance of the first pre-seizure signs (5–10 min after drug administration) or during the seizure period (15–30 min after drug administration). The bars represent mean ± S.E.M. of the utilization rate, estimated as the metabolite concentration/neurotransmitter concentration ratio. * $P \leq 0.05$ compared to the saline group; $^{+}P \leq 0.05$ compared to the vehicle group; one-way ANOVA followed by Scheffé test.

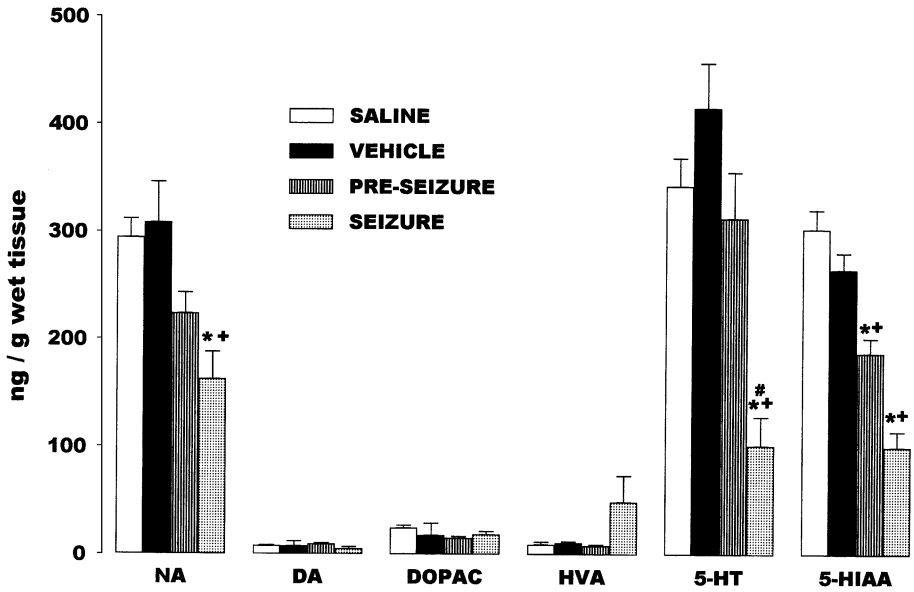

Fig. 5. Alterations in hippocampal monoamines in ricinine-treated mice sacrificed just after the appearance of the first pre-seizure signs (5–10 min after drug administration) or during the seizure period (15–30 min after drug administration). The bars represent mean ± S.E.M. monoamine concentrations. $^{*}P \leq 0.05$ compared to the saline group; $^{+}P \leq 0.05$ compared to the vehicle group; $^{\#}P \leq 0.05$ compared to the pre-seizure group; one-way ANOVA followed by Scheffé test.

$P \leq 0.001$; $P \leq 0.05$, Scheffé test], and 5-HIAA [$F(3,36) = 17.36$; $P \leq 0.001$] were decreased during the pre-seizure period; and the concentrations of NA, DA, DOPAC [$F(3,36) = 9.31$; $P \leq 0.001$], 5-HT, and 5-HIAA were significantly decreased during the seizure period (post-hoc Scheffé test, $P \leq 0.05$). The alterations in the utilization rate of DA and 5-HT in the cerebral cortex of the animals treated with ricinine are illustrated in Fig. 4. A significant increase in the DA utilization rate (HVA/DA) was observed in the cortex of the animals sacrificed during the pre-seizure and seizure periods [$F(3,35) = 8.38$; $P \leq 0.01$, one-way ANOVA; $P \leq 0.01$, Scheffé test]. Fig. 5 shows that 5-HIAA concentration was significantly decreased in the hippocampus during the pre-seizure period [$F(3,34) = 33.00$; $P \leq 0.01$; $P \leq 0.05$, Scheffé test]; and the concentrations of NA [$F(3,33) = 6.46$; $P \leq 0.001$; $P \leq 0.05$, Scheffé test], 5-HT

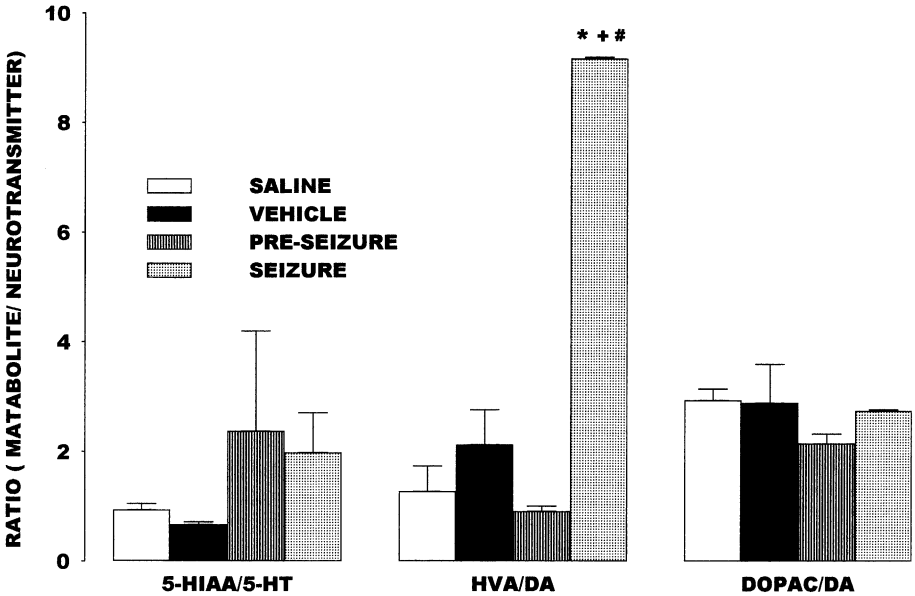

Fig. 6. Utilization rate of hippocampal monoamines in ricinine-treated mice sacrificed just after the appearance of the first pre-seizure signs (5–10 min after drug administration) or during the seizure period (15–30 min after drug administration). The bars represent mean ± S.E.M. utilization rate, estimated as the metabolite concentration/neurotransmitter concentration ratio. $^{*}P \leq 0.05$ compared to the saline group; $^{+}P \leq 0.05$ compared to the vehicle group; $^{\#}P \leq 0.05$ compared to the pre-seizure group; one-way ANOVA followed by Scheffé test.

[$F(3,35) = 14.81$; $P \leq 0.001$; $P \leq 0.05$, Scheffé test], and 5-HIAA ($P \leq 0.05$, post-hoc Scheffé test) were decreased during the seizure period. As shown in Fig. 6, ricinine administration also induced an increase in HVA/DA utilization rate in the hippocampus of the animals sacrificed during the seizure period [$F(3,12) = 57.64$; $P \leq 0.001$; $P \leq 0.001$, Scheffé test].

The vehicle used to dissolve ricinine did not affect the concentrations of the tested amino acids and monoamines in the hippocampus and cerebral cortex of the rats compared to the animals that received saline ($P \geq 0.2$, Scheffé test after ANOVA).

4. Discussion

The ricinine-elicited seizures induced significant neurochemical changes in the cerebral cortex and hippocampus of mice. The cerebral cortex showed decreased levels of Glu, Gln, and Tau during both the pre-seizure and seizure periods, suggesting an increased release of Glu and Tau by neurones and an increased consumption of Gln by glial cells involved in Glu turnover. On the other hand, no alterations in the levels of Glu or Gln were observed in the hippocampus of the animals during the preseizure or seizure periods. In contrast, the GABA level, which was not modified in the cerebral cortex during the ricinine-induced seizure, was decreased in the hippocampus of mice during the seizure period. The difference in amino acid concentration between the cortex and hippocampus may reflect the origin and direction of propagation of epileptic activity. This hypothesis was previously confirmed by the EEG recordings of mice during the ricinine-induced seizure. The EEG suggests that the seizure starts in the cerebral cortex, spreading to the hippocampus and possibly to other cerebral structures (Ferraz et al., 2000).

There is substantial evidence showing that some types of epileptic seizures can be elicited by an increased release of Glu (Akimitsu et al., 2000; Chapman, 2000; Loscher, 1998). Within this context, ricinine could be inducing seizures through Glu release, and this fact could explain the low level of this amino acid found in the cortex of ricinine-treated animals. In addition, the decreased levels of Gln in the cerebral cortex may reflect the transformation of this amino acid to Glu with later release of this neurotransmitter at the synaptic cleft level. Another possibility is a decreased activity of Gln synthetase present in glial cells.

The seizure spread mechanism proposed in the present study for ricinine has been previously reported by Sejima et al. (1997) to explain the seizures induced by pentylenetetrazole (PTZ) in rats. These authors showed that the level of Glu increased in the brainstem of PTZ-kindling rats. They interpreted this result as a suggestion that the brainstem could be in the route of the pathways for propagation of the PTZ-kindling epileptic activity, if not its primary site, and that Glu could be mediating it. Nakase et al. (1990) also showed that amygdaloid kindling in cats induces a decrease in Glu concentration in the amygdala, hippocampus, and piriform cortex during seizures. These authors also suggested that Glu was released during seizure induction. In a recent study, Li et al. (2000) observed increased Glu levels in the frontal cortex of freely moving PTZ-kindled rats using microdialysis probes, showing that this amino acid is released in the hippocampus of PTZ-kindled rats.

The decrease in GABA concentration in several tissues can be generally considered as evidence for its release at the synaptic cleft, preventing the spread of epileptic activity. The reduced concentration of GABA observed during ricinine-induced seizure in the hippocampus may be an attempt to block the seizure, which started in the cerebral cortex of ricinine-treated mice. The failure of this mechanism in the cerebral cortex would explain the more intense electrographic activity observed in the cortex when compared to the hippocampus during the initial period of the ricinine-induced seizure (Ferraz et al., 2000). It is also interesting to note that ricinine-induced seizures could be completely blocked by pre-treatment of mice with diazepam (Ferraz et al., 2000).

Another feature observed in the present study was a decreased level of Tau in the cerebral cortex of mice, possibly reflecting an increased release of Tau. These data agree with the reports of Li et al. (2000) who found an increase in Tau release by the frontal cortex of PTZ-kindled rats using in vivo microdialysis. Due to its proposed anticonvulsant action, Tau release may be another attempt to block the ricinine-elicited seizure. This idea is reinforced by some studies attributing an anticonvulsant action to this amino acid in human epilepsy (Wilson et al., 1996) and in audiogenic seizures in rats (Batuev et al., 1997). In addition, Batuev et al. (1997) also showed that intraperitoneal injection of Tau is able to block audiogenic seizures in rats of the K-M line. In another study, Loscher et al. (1993) showed decreased Tau levels in the pons and medulla of rats during seizures induced by amygdala kindling. A decrease in Tau levels was also observed in the hippocampus of rats during seizures induced by systemic administration of kainic acid (Caruso et al., 1984) or by daily electrical stimulation of the corneal pathway (Pozdeev et al., 1984).

In the present study, we also observed a significant decrease in DA concentration associated with an increased HVA/DA ratio of this monoamine in the cerebral cortex during the ricinine-elicited seizure. These data suggest an increased release of DA during seizures. DOPAC concentration also decreased in the cerebral cortex, but no significant alteration in the DOPAC/DA ratio was observed. This was probably due to the reported observation that the pathway of DOPAC formation from DA is more direct and normally more sensitive to alterations during DA turnover than the pathway of HVA formation (Altar et al., 1987). Some studies (Barone et al., 1992; Starr, 1996; Turski et al., 1990) have suggested that DA inhibits epileptic activity. Thus, the release of DA during ricinine-induced seizures

could also be part of another negative feedback mechanism inhibiting the propagation of epileptic activity. It is interesting to note that the increase in the rate of DA utilization was observed during both the pre-seizure and seizure periods in the cerebral cortex, but only during the seizure period in the hippocampus. Again, these results, taken together with previous electroencephalographic reports (Ferraz et al., 2000), suggest that the ricinine-induced seizure initiates in the cerebral cortex, where earlier and more pronounced neurochemical alterations were observed. Thus, the seizure could spread to other cerebral structures (e.g., the hippocampus).

The concentration of both 5-HT and 5-HIAA was decreased in the cerebral cortex and in the hippocampus, except for the concentration of 5-HT during the pre-seizure period, thus reinforcing the idea that epileptic activity initiates in the cerebral cortex. However, no significant difference was found concerning the 5-HIAA/5-HT utilization rate. Many studies suggest that 5-HT acting on 5-HT_{1A}, 5-HT_{1B} (Dailey et al., 1997; Statnick et al., 1996; Wada et al., 1997), and 5-HT_{2C} (Applegate and Tecott, 1998) receptors can inhibit audiogenic (Dailey et al., 1997; Statnick et al., 1996), intra-brain electrically kindled- (Applegate and Tecott, 1998; Wada et al., 1997), corneal electroshock- (Applegate and Tecott, 1998), flurothyl-, (Applegate and Tecott, 1998), PTZ- (Lazarova et al., 1984), bicuculline- (Salgado-Commissariat and Alkadhi, 1997), and intra-hippocampus kainic acid-induced seizures (Gariboldi et al., 1996). These studies used pharmacological (Dailey et al., 1997; Lazarova et al., 1984; Salgado-Commissariat and Alkadhi, 1997), genetic (Dailey et al., 1997), and neurochemical (Dailey et al., 1997) evidence for the anticonvulsant role of the serotonergic system.

The concentration of NA increased during the preseizure period in the cerebral cortex and decreased during the seizure period in the cerebral cortex and in the hippocampus, possibly due to an release followed by metabolization. NA has been proposed to have both pro- and anticonvulsant properties. Lesions of the locus coeruleus impair kindling (Corcoran and Mason, 1980) and audiogenic seizures in genetic animal models of epilepsy (Clough et al., 1994) and the restoration of NA levels by fetal locus ceruleus transplants improves kindling (Clough et al., 1994) and pilocarpine-induced seizures (Bortolotto et al., 1990). However, a study by Noebles (1984) implicated NA in the development of seizures in a genetic model of epilepsy. Rutecki (1995) and Radisavljevic et al. (1994) related the seizure-promoting effect of NA to the stimulation of β-receptors, while Rutecki (1995) associated the seizure-improving effect of NA with α-receptors. The present study suggests a recruitment of the noradrenergic system during ricinine-induced seizures, but further experiments will be necessary to elucidate its precise role.

In most of the available animal models of convulsive activity, the epileptic activity initiates in limbic structures such as the hippocampus and amygdala. These data were obtained in the pilocarpine model of epilepsy in which the epileptic activity initiates in the hippocampus of rats (Cavalheiro et al., 1994), or in the kindling model, in which the epileptic activity initiates in the amygdala (Gutierrez, 1998). In contrast to the pilocarpine, kainic acid, or kindling models, the ricinine-induced seizures are not a model of epilepsy since there is no recurrence of seizures. Nevertheless, the ricinine-induced seizures may be important to study disturbances in cortical structures that result in seizures.

In summary, the present study suggests that an imbalance between an increased release of Glu in the cerebral cortex, which is not accompanied by a significant increase in GABA release in this cerebral structure, could be implicated in the genesis of the ricinine-induced seizure. This mechanism can be aggravated by a partial postsynaptic blockage of $GABA_A$ receptors as suggested in a previous study (Ferraz et al., 2000). After the appearance of epileptic activity, many anticonvulsive mechanisms seem to be recruited, such as the release of Tau, DA, 5-HT, and NA. The fact, that the alteration in the concentration of these neurotransmitters and metabolites occurs sooner and more intensively in the cerebral cortex compared to the hippocampus, reinforces previous evidence (Ferraz et al., 2000) that the epileptic activity initiates in the cortex and spreads to other brain regions, like the hippocampus.

Acknowledgments

This work was supported by CNPq. The skilful technical assistance of Adriana Freitas in the biochemical experiments is acknowledged.

References

Akimitsu T, Kurisu K, Hanaya R, Lida K, Kiura Y, Arita K, Matsubayashi H, Ishihara K, Kitada K, Serikawa T, Sasa M. Epileptic seizures induced by *N*-acetyl-L-aspartate in rats: in vivo and in vitro studies. Brain Res 2000;861:143–50.

Altar CA, Marien MR, Marshall JF. Time course of adaptations in dopamine biosynthesis, metabolism, and a release following nigostriatal lesions: implications for behavioral recovery from brain injury. J Neurochem 1987;48:390–9.

Applegate CD, Tecott LH. Global increases in seizure susceptibility in mice lacking 5-HT_{2C} receptors: a behavioral analysis. Exp Neurol 1998;154: 522–30.

Barone P, Palma V, De Bartolomeis A, Cicarelli G, Campanella G. Dopaminergic regulation of epileptic activity. Neurochem Int 1992;20: 245–9.

Batuev AS, Bragina TA, Alexandrov AS, Riabinskaia EA. Audiogenic epilepsy: morphofunctional analysis. Zh Vyssh Nervn Deyat Im IP Pavlova 1997;47:431–8.

Bortolotto ZA, Calderazzo L, Cavalheiro EA. Some evidence that intrahippocampal grafting of noradrenergic neurons suppresses spontaneous seizures in epileptic rats. Braz J Med Biol Res 1990;23:1267–9.

Caruso G, Marano P, Patti F, De Simone D, Rafaele R, Giammona G, Nicoletti F. Effects of systemic administration of kainic acid on gamma-aminobutyric acidergic and glutamatergic transmission in various brain regions. Boll Soc Ital Sper 1984;60:1715–20.

Cavalheiro EA, Fernandes MJ, Turski L, Naffah-Mazacoratti MG. Sponta-

neous recurrent seizures in rats: amino acid and monoamine determination in the hippocampus. Epilepsia 1994;35:1–11.
Chapman AC. Glutamate and epilepsy. J Nutr 2000;130:1043–5.
Clough RW, Browning RA, Maring ML, Statinick MA, Wang C, Jobe PC. Effects of intraventricular locus coeruleus transplants on seizure severity in genetically epilepsy-prone rats following depletion of brain norepinephrine. J Neural Transplant Plast 1994;5:65–79.
Corcoran ME, Mason ST. Role of forebrain catecholamines in the amygdaloid kindling. Brain Res 1980;190:473–84.
Dailey JW, Reith MEA, Yan QS, Li MY, Jobe PC. Carbamazepine increases extracellular serotonin concentration — lack of antagonism by tetrodotoxin or zero Ca^{2+}. Eur J Pharmacol 1997;328:153–62.
Devinski O, Ehrenberg B, Bartthlen GM, Abramson HS, Luciano D. Epilepsy and sleep apnea syndrome. Neurology 1995;45:2303–4.
Farah MO, Hassan AB, Hashim MM, Atta AH. Phytochemical and pharmacological studies on the leaves of *Ricinus communis* L. Egypt J Vet Sci 1988;24:169–80.
Ferraz AC, Angelucci ME, Da Costa ML, Batista IR, Oliveira BH, Da Cunha C. Pharmacological evaluation of ricinine, a central nervous system stimulant isolated from *Ricinus communis*. Pharmacol, Biochem Behav 1999;63:367–75.
Ferraz AC, Pereira LF, Ribeiro RL, Wolfman C, Medina JH, Scorza FA, Santos NF, Cavalheiro EA, Da Cunha C. Ricinine-elicited seizures: a novel chemical model of convulsive seizures. Pharmacol, Biochem Behav 2000;65:577–83.
Gariboldi M, Tutka P, Samanin R, Vezzani A. Stimulation of 5-HT_{1A} receptors in the dorsal hippocampus and inhibition of limbic seizures induced by kainic acid in rats. Br J Pharmacol 1996;119:813–8.
Gropman AL, Barkovich AJ, Vezina LG, Conry JA, Dubovsky EC, Packer RJ. Pediatric congenital bilateral perisylvian syndrome — clinical and MRI features in 12 patients. Neuropediatrics 1997;28:198–203.
Gutierrez R. Synaptic plasticity and epileptogenesis. Salud Ment 1998; 21: 51–4.
Jacobsson SOP, Cassel GE, Karlsson BM, Sellström A, Persson SA. Release of dopamine, GABA and EAA in rats during intrastriatal perfusion with kainic acid, NMDA and soman: a comparative microdialysis study. Arch Toxicol 1997;71:756–65.
Langenerger AR, Tokarnia CH, Dobereiner J. Intoxicação experimental em coelhos com o pericarpo do fruto de *Ricinus communis*. Pesqui Vet Bras 1981;1:141–4.
Lazarova M, Bendotti C, Samanin R. Evidence that the dorsal raphe area is involved in the effect of clonidine against pentylenetetrazole-induced seizures in rats. Naunyn-Schmiedeberg's Arch Pharmacol 1984;325: 12–6.
Li Z, Yamamoto Y, Morimoto T, Ono J, Okada S, Yamatodani A. The effect of pentylenetetrazole-kindling on the extracellular glutamate and taurine levels in the frontal cortex of rats. Neurosci Lett 2000;282:117–9.
Loscher W. Pharmacology of glutamate receptors antagonists in the kindling model of epilepsy. Prog Neurobiol 1998;54:721–41.
Loscher W, Hoerstermann D, Hoenack D, Rundfelt C, Wahnschaffe U. Transmitter amino acid levels in rat brain regions after amygdala-kindling or chronic electrode implantation without kindling: evidence for a pro-kindling effect of prolonged electrode implantation. Neurochem Res 1993;18:775–81.
Meldrum BS. Neurotransmission in epilepsy. Epilepsia 1995;36:30–5.
Nakase H, Tada T, Eguchit T, Hirabayashi H, Saskai T, Enoki Y. Changes in content of excitatory amino acids in the brain and cerebrospinal fluid. Brain Nerve 1990;42:971–7.
Noebles JL. A single gene error of noradrenergic axon growth synchronizes central neurons. Nature 1984;310:409–11.
Olsen RW, Avoli M. GABA and epileptogenesis. Epilepsia 1997;38: 399–407.
Pozdeev VK, Gracheva GM, Ilin AP. The effect of electrical stimulation in the kindling regimen on transmitter systems of the rat brain. Fiziol Zh Sssr Im IM Sechenova 1984;70:944–51.
Radisavljevic Z, Cepeda C, Peacock WC, Buchwald NA, Levine MS. Norepinephrine modulates excitatory amino acid-induced responses in developing human and adult rat cerebral cortex. Int J Dev Neurosci 1994; 12:353–61.
Rutecki PA. Noradrenergic modulation of epileptiform activity in the hippocampus. Epilepsy Res 1995;20:125–36.
Salgado-Commissariat D, Alkadhi KA. Serotonin inhibits epileptiform discharge by activation of 5-HT_{1A} receptors in CA1 pyramidal neurons. Neuropharmacology 1997;36:1705–12.
Sejima H, Ito M, Kishi K, Tsuda H, Shiraishi H. Regional excitatory and inhibitory amino acid concentrations in pentylenetetrazole kindling and kindled rat brain. Brain Dev 1997;19:171–5.
Starr MS. The role of dopamine in epilepsy. Synapse 1996;22:159–94.
Statnick MA, Dailey JW, Jobe PC, Brwoning RA. Abnormalities in 5-HT_{1A} and 5-HT_{1B} receptor binding in severe-seizure genetically epilepsy-prone rats (GERPR-9s). Neuropharmacology 1996;35:111–8.
Tokarnia CH, Döbereiner J, Da Silva MF. Plantas Tóxicas da Amazônia a Bovinos e outros Herbívoros. Manaus: INPA, 1979.
Turski WA, Cavalheiro EA, Ikonomidou C, Bortolotto ZA, Klockgether T, Turski L. Dopamine control of seizure propagation: intranigral dopamine D1 agonist SKF-38393 enhances susceptibility to seizures. Synapse 1990;5:113–9.
Wada Y, Shiraishi J, Nakamura M, Koshino Y. Role of serotonin receptor subtypes in the development of amygdaloid kindling in rats. Brain Res 1997;747:338–42.
Wilson CL, Maidment NT, Shomer MH, Behnke EJ, Ackerson L, Fried I, Engel J. Intrahippocampal or intra-amygdala infusion of Kn62, a specific inhibitor of calcium/calmodulin-dependent protein kinase II, causes retrograde amnesia in the rat. Behav Neural Biol 1996;61: 203–5.