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Study pharmacologic of the GABAergic and glutamatergic drugs on seizures and status epilepticus induced by pilocarpine in adult Wistar rats

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Abstract

This work was designed to study the influence of drugs during seizures and status epilepticus (SE) induced by pilocarpine and mortality in adult rats. Glutamate (10 and 20 mg/kg), *N*-methyl-D-aspartate (NMDA, 5 and 10 mg/kg), ketamine (1.5 and 2.0 mg/kg), gabapentin (200 and 250 mg/kg), phenobarbital (50 and 100 mg/kg) and vigabatrine (250 and 500 mg/kg) were administered intraperitoneally, 30 min prior to pilocarpine (400 mg/kg, i.p.). The animals were observed (24 h) to determine: number of peripheral cholinergic signs, tremors, stereotyped movements, seizures, SE, latency to first seizure and number of deaths after pilocarpina treatment. NMDA and glutamate had pro-convulsive effects in both doses tested. Smaller and higher doses of these drugs no protected and increased pilocarpine-induced seizures and/or mortality. Gabapentin, vigabatrine, phenobarbital and ketamine protected against seizures and increased the latency to first seizure. Thus, these results suggest that caution should be taken in the selection of pharmacotherapy and dosages for patients with seizures and SE because of the possibility of facility the convulsive process toxicity, SE and the mortality of adult animals in this seizures model that is similar temporal lobo epilepsy in humans.

Keywords: Pilocarpine; Seizures; Status epilepticus; NMDA; Glutamate; Ketamine; Phenobarbital; Gamma amino butyric acid pentin; Vigabatrine

Pilocarpine-induced seizures and status epilepticus (SE) produces many alterations in central nervous system neurotransmission. Previous work from our laboratory has demonstrated that intraperitoneal administration of pilocarpine 400 mg/kg significantly decreases not only muscarinic M_1, M_2 and GABAergic receptors densities, but also decrease acetylcholinesterase (AChE) and increases superoxide dismutase and catalase enzymatic activities in the rat striatum, frontal cortex and hippocampus [14,16]. Recently, it was observed that the acute treatment with 400 mg/kg pilocarpine induces long-lasting alterations in serotonergic and glutamatergic receptors in rat frontal cortex, hippocampus and striatum [19,33].

Data in the literature indicate that the changes produced by epileptic activity is related to the development of acute symptoms (peripheral cholinergic signs, tremors, staring spells, facial automatisms and motor seizures, which develop progressively within 1–2 h into a long-lasting SE) or diseases (psychopathologies associated with seizures, such as depression, anxiety, irritability, sleep problems, paranoia and mania). These conditions can be treated with medications that are also can be used during seizures [33,17,18,15].

Seizures and SE has medical consequences with respect to morbidity and mortality of 0.5% prevalence [1]. One of the most important manifestations of pilocarpine treatment is the occurrence of seizures, status epilepticus and mortality. Epilepsy animal models have demonstrated that convulsions induced by pilocarpine are relatively insensitive to standard anticonvulsant therapies [4] and [11] and the mechanism of pilocarpine-induced seizures has not been fully elucidated. Pharmacological studies [23] have demonstrated the involvement of Gabaergic receptors,

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as treatment with diazepam and phenobarbital, an inhibitor of gamma amino butyric acid uptake, protects against pentylenetetrazole clonic seizures in mice [33]. NMDA receptor antagonists are also effective, whereas sodium and calcium channel blockers are ineffective [23]. Involvement of gamma amino butyric acid (GABA), adenosine, glutamate, aspartate, tyrosine, taurine, serotonine, dopamine and norepinephrine neurotransmitters has also been proposed [22,3,5]. Relative to these data, we recently observed significant alterations in monoamine (dopamine, serotonin and norepinephrine) and their metabolites (DOPAC, HVA and 5-HIAA) levels in rat striatum, hippocampus and frontal cortex after pilocarpine-induced status epilepticus of adult rats [10,8,20].

Based on this evidence, we designed this study to determine the influence of drugs used to treat pathologies associated pilocarpine-induced seizures and SE, such as GABAergic and glutamatergic drugs, on pilocarpine-induced seizures, SE and mortality in adult rats and to determine the possible risks and/or benefits resulting from exposure of addicts to these medications and the incidence of relapse to seizures and SE. The purposed is investigated drugs that can induce other alterations in cerebral mechanisms and possibly contribute for prevent of the neuronal damage and mortality rate of young and adult epileptic patients through of study in epilepsy models similar the temporal lobo epilepsy in humans.

Adult Wistar male rats (250–280 g) maintained in a temperature-controlled room (26 °C) with a 12-h light/dark cycle with food and water ad libitum was used. All experiments were performed according to the Guide for the Care and Use of Laboratory Animals from the US Department of Health and Human Services [32], and the project was approved by the Committee of Ethics in Animal Research, Department of Physiology and Pharmacology, Faculty Catholic of Rainha of Sertão, Ceará, Brazil.

The following substances were used: pilocarpine hydrochloride, *N*-methyl-D-aspartate (NMDA), glutamate (Sigma Chemical, USA), phenobarbital (Sigma Chemical, USA), vigabatrine (Sigma Chemical, USA), Gabapentin (Neurontin, Parke-Davis, Freiburg, Germany) and ketamine (Francotar, Virbac, Brazil).

All doses are expressed in milligrams per kilogram and were administered in a volume of 10 ml/kg injected intraperitoneally (i.p.). Rats were pretreated with one of several doses of glutamate (10 and 20 mg/kg), phenobarbital (50 and 100 mg/kg), NMDA (5 and 10 mg/kg), gabapentin (200 and 250 mg/kg), vigabatrine (500 and 750 mg/kg) and ketamine (1.5 and 2.0 mg/kg), 30 min prior to intraperitoneal administration of pilocarpine 400 mg/kg, and in this 30-min interval rats were observed for the occurrence of any change in behavior. After pilocarpine injection, the behavioral alterations of the animals were observed in 30 cm × 30 cm chambers to record: latency to the first seizure (any one of the behavioral indices typically observed after pilocarpine administration during 24 h such as peripheral cholinergic reactions (miosis, piloerection, chromodacryorrhea, diarrhea, masticatory), tremors and stereotyped movements, generalized clonic–tonic seizures, latency to the development of the first seizure, status epilepticus and mortality rate [8], number of animals that seized, and number of animals that died after pilocarpine administration.

The drug dosages were determined from both dose–response studies, including pilocarpine (data not shown), and observations of the doses currently used in animal studies in the literature. The doses used are not equivalent to those used by humans because rats have higher metabolic rates.

Results of the latency to first seizure were compared using ANOVA and the Student–Newman–Keuls test as *post hoc* test, because these results show a parametric distribution. In this situation statistical significance was reached at p less-than-or-equals, slant 0.05.The number of animals that seized and the number that survived were calculated as percentages (% seizures and % survival, respectively) and compared with a non-parametric test (χ^2). In both situations statistical significance was reached at p less-than-or-equals, slant 0.0001. The statistical analyses were performed with the software GraphPad Prism, Version 3.00 for Windows, GraphPad Software (San Diego, CA, USA).

Pilocarpine (400 mg/kg, i.p.) induced the first seizure at min. All the 80 animals studied showed generalized clonic tonic convulsions with status epilepticus, and 27% survived the seizures.

Table 1
Effect of pretreatment with glutamatergic drugs on pilocarpine-induced seizures and lethality in adult rats

Pharmacological class	Drugs	Dose (mg/kg)	Latency to first seizures (min)	Seizures (%)	Survival (%)	Number of animals/group
Glutamatergic drugs	Pilocarpine	400	35.00 ± 0.70	75	27	80
	NMDA	5	27.75 ± 2.90*	100[a]	00[a]	16
		10	20.65 ± 2.95*	100[a]	00[a]	16
	Glutamate	10	11.75 ± 0.90*	100[a]	00[a]	16
		20	9.85 ± 0.95*	100[a]	00[a]	16
	Ketamine	1.5	97.75 ± 1.90*	34[a]	64[a]	16
		2.0	101.55 ± 2.75*	26[a]	74[a]	16

Animals were pretreated acutely, intraperitoneally, with drugs listed above and 30 min after receiving pilocarpine 400 mg/kg, intraperitoneally. Results for latency to first seizure are expressed as means ± S.E.M. of the number of experiments shown in the table. Result for % seizures and % survival are expressed as percentages of the number of animals from each experimental group.

[a] $p < 0.0001$ as compared with pilocarpine group (χ^2 test).

* $p < 0.0001$ as compared with pilocarpine group (ANOVA and Student–Newman–Keuls test).