

THE CONTRIBUTION OF THE LATERAL POSTERIOR AND ANTEROVENTRAL THALAMIC NUCLEI ON SPONTANEOUS RECURRENT SEIZURES IN THE PILOCARPINE MODEL OF EPILEPSY

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ABSTRACT - The pilocarpine model of epilepsy in rats is characterised by the occurrence of spontaneous seizures (SRSs) during the chronic period that recur 2-3 times per week during the whole animal life. In a previous study on brain metabolism during the chronic period of the pilocarpine model it was possible to observe that, among several brain structures, the lateral posterior thalamic nuclei (LP) showed a strikingly increased metabolism. Some evidences suggest that the LP can participate in an inhibitory control system involved in the propagation of the seizures. The aim of the present study was to verify the role of LP in the expression and frequency of spontaneous seizures observed in the pilocarpine model. Ten adult male rats presenting SRSs were monitored for behavioural events by video system one month before and one month after LP ibotenic acid lesion. Another group of chronic epileptic rats (n=10) had the anteroventral thalamic nuclei (AV) lesioned by ibotenic acid. After the surgical procedure, the animals were sacrificed and the brains were processed for histological analysis by the Nissl method. The LP group seizure frequency was 3.1 ± 1.9 before ibotenic acid injection and showed an increase (16.3 ± 7.2 per week) after LP lesion. No changes in SRSs frequency were observed in the AV group after ibotenic lesion in these nuclei. These results seem to suggest that LP play a role in the seizure circuitry inhibiting the expression of spontaneous seizures in the pilocarpine model.

KEY WORDS: pilocarpine, lateral posterior thalamic nuclei.

A contribuição dos núcleos talâmico lateral posterior e anteroventral nas crises espontâneas e recorrentes no modelo de epilepsia induzido pela pilocarpina

RESUMO - O modelo de epilepsia induzido pela pilocarpina é caracterizado pela ocorrência de crises espontâneas e recorrentes (CERs) durante o período crônico, em uma frequência de 2-3 episódios semanais durante toda a vida do animal. Um estudo prévio do metabolismo cerebral durante o período crônico do modelo da pilocarpina mostra que entre as diferentes estruturas cerebrais, o núcleo talâmico lateral posterior (LP) apresentou um significativo aumento em seu metabolismo. Evidências sugerem que o LP pode participar como um sistema de controle inibitório na propagação das crises. O objetivo deste estudo foi verificar o papel do LP na expressão e frequência das CERs no modelo de epilepsia induzido pela pilocarpina. Dez ratos machos adultos da raça Wistar que apresentaram CERs, foram monitorados por um período de dois meses, sendo um mês antes e outro após a lesão do LP com ácido ibotênico, através de um sistema de vídeo para verificar os eventos comportamentais. Outro grupo de animais epiléticos crônicos (n=10) sofreu lesão do núcleo talâmico anteroventral (AV) pelo ácido ibotênico. Após esses procedimentos, os animais foram sacrificados e realizada a análise histológica cerebral através do método de Nissl. O grupo LP, o qual apresentava $3,1 \pm 1,9$ crises por semana antes de sofrer a lesão por ácido ibotênico, apresentou aumento na frequência das CERs de 50,5% após a lesão ($16,3 \pm 7,2$ CERs por semana). Não foi observada alteração na frequência de CERs nos animais pertencentes ao grupo AV. Nossos resultados sugerem que o LP está implicado no mecanismo de controle inibitório das CERs no modelo de epilepsia induzido pela pilocarpina.

PALAVRAS-CHAVE: pilocarpina, núcleo talâmico lateral posterior.

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Seizures may arise from an hyperexcitable network in different cerebral areas defined as epileptic foci. Limbic motor seizures rely on forebrain structures for their initiation and propagation¹. The mechanisms regulating the expression of epileptogenic properties in such areas have been investigated on the last decades since it could determine specific approaches for the control of epileptic phenomena. Several structures have been considered as inhibitory sites for seizure generation. These "seizure regulatory zones" may be relevant for seizure origin and propagation but may also be only indirectly involved in the mechanisms of seizure arrest². The possible role of thalamic nuclei in triggering and spreading of epileptic discharges have been discussed over the last four decades since the previous work of Jasper and his collaborators³. The importance of the thalamus in the genesis of epileptic seizures obviously correlates with its extensive projection to the cortex and other areas⁴.

The pilocarpine model of epilepsy has been largely used to study the pathophysiology of epilepsy since its main features resemble those seen in human temporal lobe epilepsy. The systemic administration of pilocarpine (PILO), a potent muscarinic agonist, to rats promotes an acute sequence of behavioural and electrographic changes that built up progressively into limbic status epilepticus and lasts 24h, called of acute period. After that a silent period is observed with progressive normalisation of EEG and behaviour which varies from 4 to 44 days followed by a chronic period, characterised by spontaneous recurrent seizures (SRSs)⁵. The main features of the SRSs observed during the long-term period resemble those of human complex partial seizures and recur 2-3 times per week per animal. Therefore, the pilocarpine model of epilepsy provides an unique experimental condition for studying the human disorder.

Scorza and collaborators⁶ studying the interictal cerebral metabolic rate by ¹⁴C-2DG autoradiography in chronic PILO-induced rats, found an increase in glucose utilization by several epileptic brain regions. The most relevant finding was a consistent rise of cerebral metabolic rate in lateral posterior thalamic nuclei (LP), suggesting that LP may be involved in the cerebral circuitry controlling epileptic activity during interictal intervals. The lateral posterior thalamic nuclei is a single homogeneous region in the dorsal aspect of the thalamus, intercalated between the dorsal lateral geniculate nuclei, the medial region of the posterior nuclei and the central lateral nuclei. It extends posteriorly between the pretectal area and the dorsal medial geniculate nuclei⁷. The lateral posterior thalamic nuclei receives fibers from several vi-

sion-related structures, including the superior colliculus, pretectal nuclei, and ventral lateral geniculate nuclei, and has extensive connections with parts of the hippocampal formation⁸. On the other hand, the anterior thalamic nuclei (AV) have afferent and efferent connections with limbic areas^{9,10}. The AV receive afferents from the hippocampus, cingulate cortex and mammillary complex of the hypothalamus. They project to the medial (cingulate) cortex and also to the presubiculum. These various connections of the AV complete circuits linking the hippocampus and hypothalamus with the cingulate cortex and represent an elaboration on the so-called "Papez circuit". The AV, therefore, form an important subcortical component of the limbic system, being important to address the question whether it can participate in the seizure spread as well as in seizure control.

To ascertain the LP or AV contribution on SRSs activity induced by pilocarpine in rats, we compared the frequency of spontaneous seizures before and after ibotenic acid lesions within these thalamic nuclei.

METHOD

Seventy adult male rats, weighting 280-300g were housed under standard controlled conditions (7:00 A.M./7:00 P.M. light/dark cycle; 20-22°C; 45-55% humidity) with food and water *ad libitum*. SRSs were induced according to the procedure previously described⁵. Accordingly, 30 minutes after methylscopolamine injection (1mg/kg s.c.) pilocarpine was administrated (350 mg/kg i.p) to rats. The surviving animals (n=40) were then allowed to advance through the acute and silent periods to the chronic phase⁵.

Chronic epileptic rats were anaesthetized (thionebutal 40 mg/kg, i.p.) and given bilateral infusions over 5 min of 10 µg of ibotenic acid (Sigma)¹¹ dissolved in 0.5 µl of phosphate-buffered saline (pH 7.4) aimed to the lateral posterior thalamic nuclei (n=10) (A +3.8, L ±1.7, H -5.7)¹² or to the anteroventral thalamic nuclei (n=10) (A +1.8, L ±1.7, H -5.5)¹². The sham group, composed by chronic epileptic rats received phosphate-buffered saline (pH 7.4) according the same procedures described to the experimental group, (LP: n=10 and AV: n=10).

After the status epilepticus period, the surviving animals were continuously monitored during 24 h for the detection of spontaneous seizures, using a video system one month before and one month after surgery proceedings as described above. Infrared emitting lights were used during the dark periods to allow video recording of the animal activity during this time. To determine the number of seizures during this period two observers were recruited for all the behaviour analysis.

In order to confirm the thalamic lesions, the brains were histologically processed 30 days after ibotenic acid injection. Rats were deeply anaesthetised with an overdose of sodium pentobarbital and perfused through the

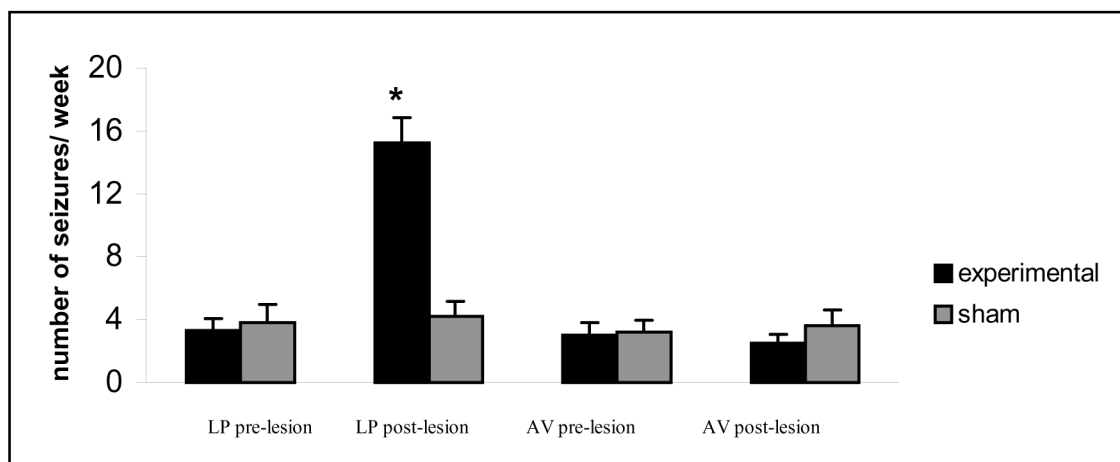


Fig 1. Graphic representation of the cumulative frequency of spontaneous seizures per week detected in sham and experimental groups, before and after saline or ibotenic acid injections into LP and AV thalamic nuclei. Data are expressed as mean \pm SD. * $p < 0.05$.

heart with saline followed by 10% formalin solution. The brains were removed, stored in formalin, embedded in paraffin and coronally sectioned every 20 μ m. Every 10th sections were preserved and stained with cresyl violet.

RESULTS

All the ibotenic acid-treated animals tolerated well the thalamic lesions. Although initially their food intake decreased, by the end of the first week they had reassumed their normal feeding patterns. On the other hand, the animals that received phosphate-buffered saline pH 7.4 into the same thalamic nuclei showed a normal behaviour.

The pattern of behavioural features, the duration and the intensity of seizures observed in this study were the same for all animals. Spontaneous recurrent seizures were characterised by facial automatism, forelimb clonus, and rearing followed by loss of postural control and generalised clonic seizures lasting 25-35 sec. The sham group did not present any change in the frequency of seizures that recurred 2-3 times per week per animal. In contrast, after ibotenic acid injection in the LP nuclei, the frequency of seizures were approximately 5 times greater than that observed before injection. On the other hand, SRSs frequency in the AV lesioned animals remained unaltered (Fig 1).

In the ibotenic acid experiments, the injected areas were optimally placed in both locations, and they were both relatively large. Some animals used in this study presented injected areas more restricted than those described above. However, a positive correlation between the lesion extension and frequency of seizures was not observed. Figure 2 shows a diagram-

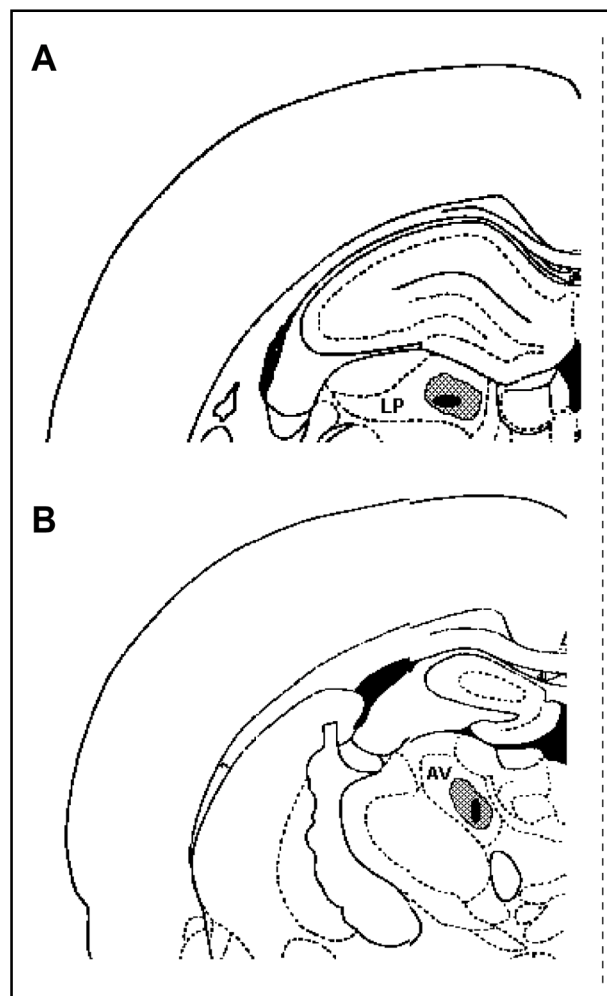


Fig 2. Diagrammatic representation of the extent of the lesioned areas following ibotenic acid injection in (A) lateral posterior thalamic nuclei and (B) anteroventral thalamic nuclei. The hatched areas represent the larger lesions while black areas depict the smaller lesions observed in the whole group of animals.

matic representation of the extent of the lesioned areas following ibotenic acid injection at the lateral posterior thalamic nuclei (A) and anteroventral thalamic nuclei (B). The hatched areas represent the larger lesions while black areas depict the smaller lesions observed in the whole group of animals. The neuronal cells of LP and AV were intact after phosphate-buffered saline injection dose.

DISCUSSION

The present study evaluates the contribution of LP and AV on SRSs activity induced by pilocarpine. The increased metabolic rate in LP during the interictal period may be a result of the activation of cerebral circuits controlling SRSs initiation and/or generalization⁶. The chronic epileptic conditions may be associated with increased inhibitory influences during the interictal state, which could reflect reactive homeostatic mechanisms to prevent seizure initiation or restrict ictal propagation once epileptogenesis occurs¹³.

The anatomic substrates activated during interictal intervals differ from those activated during seizure activity in the pilocarpine model of epilepsy. Therefore, these differences may be particularly important for understanding the triggering and spreading mechanisms underlying epileptic activity during status epilepticus and recurrent seizures⁶. Our results reinforce those considerations since we showed that LP lesion by ibotenic acid in chronic rats results in an increase of seizures frequency. Moreover, these results pointed to LP one of the most important thalamic nuclei involved in the inhibition of spreading mechanisms. Despite the AV contribution to the absence of epilepsy and its connections to the limbic system we did not observe significant changes in seizure frequency after AV lesion by ibotenic acid.

A few numbers of investigators have considered the contribution of the posterior thalamic nuclei to epilepsy. In artificially ventilated rats, an epicortical penicillin focus led to a higher uptake of glucose, especially within the posterior thalamus compared to the ventrolateral, venteropostero-lateralis or medialis areas¹⁴. Increased GABAergic transmission by a local injection of muscimol into the posterior nuclei suppressed generalised convulsive seizures in the spontaneously epileptic Mongolian Gerbil¹⁵. Besides their epileptogenic properties¹⁶, thalamic areas are also able to reduce epileptiform activity if they were stimulated electrically. Despite some encouraging findings by Velasco et al.¹⁷, thalamic stimulation in humans is still controversial.

In cats, epileptiform activity induced by local application of penicillin in the visual or motor cortex was suppressed by electrical stimulation of the medio-central, lateral geniculate, or ventrolateral thalamic nuclei¹⁸. Therefore, thalamic areas may be involved in the formation of an autoprotective mechanism, defending the brain against excessive hyperexcitation.

Our data further highlights that several thalamic nuclei may participate in different ways in the control of limbic seizures spreading and that LP particularly exerts a potent role in cerebral circuitry control of SRSs initiation and/or generalization. The exact pathways by which the lateral posterior thalamic nuclei exert their different effects in seizures are not fully understood but the extensive connections with parts of the hippocampal formation represent important relays. Our model provided directions for future research.

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