GLUCOSE UTILISATION DURING STATUS EPILEPTICUS IN AN EPILEPSY MODEL INDUCED BY PILOCARPINE

A qualitative study

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ABSTRACT - Status epilepticus (SE) is a medical emergency and it is associated to brain damage. 2-deoxy-[^14]C glucose (2-DG) procedure has been used to measure the alterations in the functional activity of the brain induced by various pharmacological and toxicological agents. The aim of this study was to determine which changes occur in the seizure anatomic substrates during the SE induced by pilocarpine (PILO) using [¹⁴C]-2 deoxyglucose functional mapping technique. Wistar male adult rats were submitted to SE PILO-induced for 6h and received [¹⁴C] 2-deoxyglucose injection via jugular vein 45 min before the 6th hour of SE. The control animals were submitted to all procedures but received saline and not pilocarpine. Brain sections were prepared and exposed X-ray film about seven days. The optical density of each region was obtained using a solid state digital analyser. The analysis revealed that [¹⁴C]-2DG utilisation was pronounced in the SE rats on the areas corresponding to the hippocampal formation (+50.6%), caudate-putamen (+30.6%), frontoparietal cortex (+32.2%), amygdala (+31.7%), entorinal cortex (+28.2%), thalamic nucleus (+93.5%), pre-tectal area (+50.1%) and substantia nigra (+50.3%) when compared to control. Our results suggest that the different activation levels of the distinct structures may be particularly important for understanding triggering and spreading mechanisms underlying epileptic activity during status epilepticus.

KEY WORDS: deoxyglucose, pilocarpine, seizure, status epilepticus.

Utilização de glicose durante o estado de mal epiléptico no modelo de epilepsia induzido pela pilocarpina: um estudo qualitativo

RESUMO - O estado de mal epiléptico (SE) é uma emergência médica e está associado a lesão cerebral. O procedimento da [¹⁴C] desoxiglicose tem sido utilizado para avaliar as alterações da atividade funcional cerebral induzidas por agentes farmacológicos e toxicológicos. O objetivo deste estudo foi verificar as alterações metabólicas do cérebro de ratos durante o SE induzido pela pilocarpina, para tanto, utilizamos a técnica de mapeamento funcional da [¹⁴C] desoxiglicose. Ratos machos da raça Wistar foram submetidos ao SE induzido pela pilocarpina durante período de 6 horas; 45 minutos antes de se completar 6 horas de SE, tais animais receberam uma injeção de [¹⁴C] desoxiglicose por via venosa (veia jugular). Os animais pertencentes ao grupo controle foram submetidos aos mesmos procedimentos, no entanto, receberam solução salina e não pilocarpina. As fatias cerebrais foram preparadas e expostas em filme de raioX por um período de sete dias. A análise da densidade óptica de cada região foi obtida por analisador digital de estado sólido. Tal análise revelou aumento no consumo de glicose durante o SE nas seguintes regiões: formação hipocampal (+50,6%), núcleo caudado-putamen (+30,6%), córtex frontoparietal (+32,2%), amígdala (+31,7%), córtex entorinal (+28,2%), complexo talâmico (+93,5%), área pré-tectal (+50,1%) e substância negra (+50,3%), quando comparados com os animais pertencentes ao grupo controle. Nossos resultados sugerem que a ativação dessas estruturas deve ser particularmente importante nos mecanismos de desencadeamento e alastramento da atividade epiléptica durante o estado de mal epiléptico.

PALAVRAS-CHAVE: desoxiglicose, pilocarpina, crises epilépticas, estado de mal epiléptico.
Status epilepticus (SE) has been defined as recurrent epileptic seizures without full recovery of consciousness before the next seizure beginning, or continuous clinical and/or electrical seizure activity lasting for more than 30 minutes whether or not consciousness is impaired. The fundamental pathophysiology of SE involves a failure of mechanisms that normally abort an isolated seizure. This failure can arise from abnormally persistent, excessive excitation or ineffective recruitment of inhibition. The relative contribution of these factors is poorly understood. The temporal and spatial determinants of SE are also relatively unknown; experimental studies suggest that there is induction of reverberating seizure activity between, for example, hippocampal and parahippocampal structures and that seizures progress through a sequence of a distinct electrographic changes.

It is likely that numerous mechanisms are involved, depending on the underlying cause. Our best insights come from cases in which SE was caused by an exogenous toxin. The most notable example involved the ingestion in 1987 of mussels contaminated with domoic acid, an analogue of glutamate. Some patients had prolonged and profound SE. This occurrence suggests that excessive activation of excitatory amino acid receptors can cause prolonged seizures and suggests that excitatory amino acids have a causative role in SE. SE can also be caused by penicillin and related compounds that antagonise the effects of γ-aminobutyric acid (GABA). Engel (1995) suggests that the failure of inhibition may be due in some cases to a shift in the functional properties of the GABAergic receptor that occurs, as seizures become prolonged.

SE lasting approximately 30 to 45 minutes can cause cerebral injury, specially in limbic structures such as the hippocampus, and seizure activity itself is sufficient to damage the central nervous system (CNS). This damage is partially a consequence of glutamate-mediated excitotoxicity and does not appear to be due primarily to an excessive metabolic demand imposed by repetitive neuronal firing. The superimposition of systemic stresses such as hyperthermia, hypoxia or hypotension exacerbates the degree of neuronal injury in animal models of SE, a finding consistent with empirical observations in humans.

Alterations in the functional activity of the brain induced by various pharmacological and toxicological agents can be measured by the 2-deoxy-[14C] glucose (2-DG) procedure developed by Sokoloff et al. in 1977. The autoradiographic technique has been used to trace regional glucose utilisation by the brain tissue in normal conscious state under physiological and pathological experimental conditions.

The measurement of brain metabolism during seizures and interictal periods has been used to identify the CNS structures responsible for the generation, propagation and control of the epileptic activity. Local cerebral glucose utilisation has been shown to change dramatically during SE, and marked regional hypermetabolism has been shown to correlate with the development of neuronal damage in various models of seizures in adult rodents and primates. In the amygdaloid kindling, a normal deoxyglucose uptake without any behavioural seizure activity (e.g. in the initial stage of the kindling process) was described. However, rats with generalised motor seizures (final stage of kindling) exhibited an increase in deoxyglucose uptake by the substantia nigra, rostral globus pallidus and neocortex. Electrical stimuli to amygdala induces both an enhanced

### Table 1. Basal levels of cerebral energy metabolism of the status epilepticus of adult wistar rats.

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Control Animals (n=10)</th>
<th>Status Epilepticus Animals (n=10)</th>
<th>Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caudate-Putamen</td>
<td>163.8 ± 2.8</td>
<td>*214.7 ± 5.9</td>
<td>31.07</td>
</tr>
<tr>
<td>Frontoparietal Cortex</td>
<td>166.3 ± 2.4</td>
<td>*220.0 ± 3.6</td>
<td>32.29</td>
</tr>
<tr>
<td>Thalamic Nucleus</td>
<td>102.6 ± 2.9</td>
<td>*198.6 ± 2.4</td>
<td>93.56</td>
</tr>
<tr>
<td>Pre-Tectal Area</td>
<td>137.3 ± 3.2</td>
<td>*206.2 ± 3.9</td>
<td>50.10</td>
</tr>
<tr>
<td>Substantia Nigra</td>
<td>132.2 ± 3.0</td>
<td>*198.8 ± 2.6</td>
<td>50.30</td>
</tr>
<tr>
<td>Hippocampal Formation</td>
<td>140.7 ± 5.1</td>
<td>*212.6 ± 3.7</td>
<td>50.60</td>
</tr>
<tr>
<td>Amygdala</td>
<td>133.8 ± 2.8</td>
<td>*176.3 ± 3.3</td>
<td>31.70</td>
</tr>
<tr>
<td>Entorhinal Cortex</td>
<td>139.6 ± 3.7</td>
<td>*179.1 ± 3.6</td>
<td>28.20</td>
</tr>
</tbody>
</table>

Values are expressed as optical density (O.D). * P< 0.001, statistically significant difference from control (Unpaired Student T test).
deoxyglucose uptake and co-localised seizure activity that initially spreads from the stimulated site to a restricted circuitry and later involves the whole system. Several alterations in the local deoxyglucose uptake induced by kainic acid, bicuculine and metrazol-induced seizures were described in rats. On the other hand, in patients with temporal lobe epilepsy, the EEG, neuropathological and positron emission tomography (PET) studies have all emphasised the primary involvement of the anterior hippocampal formation and the amygdaloid complex in the pathogenesis of most complex partial seizures.

The systemic administration of a potent muscarinic agonist pilocarpine (PILO) in rats promotes a sequential behavioural and electrographic changes that can be divided into three distinct periods: (a) an acute period that built up progressively into limbic SE and that lasts 24h, (b) a silent period with a progressive normalisation of EEG and behaviour which varies from 4 to 44 days, and (c) a chronic period with spontaneous recurrent seizures (SRSs). These spontaneous seizures recur 3-5 times per week per animal and its main features resemble those of human complex partial seizures. Therefore, the pilocarpine model of epilepsy provides unique experimental conditions for studying the human disorder.

The purpose of the present study was to determine with the 14C-2 deoxyglucose functional mapping technique which changes occurs in the seizure anatomic substrates during the SE induced by pilocarpine.

**METHOD**

Adult male Wistar rats (n=40), 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. The animals were separated in two groups: a) 6h SE (n=10) and b) Control group (n=10). All the animals were anaesthetised with a pentobarbital/chloral mixture. A polyethylene catheter (PE-10, Clay Adams) was permanently implanted into one external jugular vein. To minimise the stress and facilitate the injection of 14C-2DG, the catheter was fixed over the skull and often flushed with heparinized saline after the surgical procedure. After 3h recovery from anesthesia, SE was induced by pilocarpine injection.

14C-2DG (50-55 µCi/mol; Sigma) was prepared in ethanol, evaporated under N2 stream and resuspended in normal saline. 14C-2DG (200 µg/kg) was administrated by rapid intravenous infusion. The animals were killed 45 minutes after the 14C-2DG infusion by decapitation and the brains were rapidly removed, frozen in cooled methylbutane (-40°C), and stored at -70°C.

For autoradiographical analysis, 20 µm thick coronal brain sections were prepared in cryostat (Jung CM 1800-Leica) at -20°C. Brain sections were mounted on cover slips and dried immediately at 55°C on a hot plate. Autoradiograms were then prepared and exposed to a Kodak SB-5 X-ray film (Sigma Chemical Company) for about seven days. Then, the film was processed in a developer solution (Kodak, GB). Cerebral glucose utilisation rate by different structures was evaluated through the analysis of radioactive labelling on the autoradiograms. The optical density of each region were obtained using a solid state digital analyser consisting of a charge-coupled device scanner and a computer (NIH Image version 1.57, Scanner-Epson ES 1000C).

Analysis of glucose uptake were determined in the mainly regions involved in the pathophysiology of this epilepsy model, hippocampal formation, caudate-putamen, frontoparietal cortex, amygdala, hypothalamus, entorhinal cortex, thalamic nucleus, endopiriform nucleus, primary olfactory cortex, pre-tectal area, substantia nigra.

Differences in deoxyglucose uptake between pilocarpine-treated and control animals were statistically evaluated with the Student’s T-test for p < 0.05.

**RESULTS**

Pilocarpine administration induced both ictal and interictal epileptic activity in hippocampal and cortical electrographic recordings which was correlated with the sequence of behavioural alterations, as previously described. In summary, 2-10 minutes after pilocarpine injection, a predominant theta rhythm could be observed in the hippocampal recording accompanied by slow-voltage fast activity in the cortex. Subsequent high-voltage fast activity and isolated spikes were seen in the hippocampus, which spread rapidly to the cortex leading to the synchronisation of both recordings. This kind of activity evolved to a pattern of isolated electrographic seizures, which culminated in SE.

At the same time, behavioural changes could be observed. Immediately after pilocarpine administration, animals started to show akinesia, ataxic lurching and facial automatism. This behaviour progressed to motor limbic seizures, which culminated in SE 20-50 minutes pilocarpine injection.

On the other hand, the cerebral glucose-utilisation rate measured on labelled brain structures did not differ significantly among control animals. Qualitatively, these results resembled those reported by Sokoloff et al. In contrast to the animals of control group, 14C-2DG utilisation was pronounced in the SE rats on the areas corresponding to the hippocampal formation (+50.6%), caudate-putamen (+30.6%), frontoparietal cortex (+32.2%), amygdala (+31.7%), entorhinal cortex (+28.2%), thalamic nucleus (+93.5%), pre-tectal area (+50.1%) and substantia nigra (+50.3%) (Figure).
DISCUSSION

The present study evaluates cerebral metabolic rate in rats during the acute period of the pilocarpine model of epilepsy by $^{14}$C-2DG autoradiography. The observation of increased glucose utilisation by the hippocampal formation, caudate-putamen, frontoparietal cortex, amygdala, entorhinal cortex, thalamic nucleus, pre-tectal area and substantia nigra suggests that these areas, contiguously and intensely activated, have a strong tendency to act together as a single functional entity.

In animals, investigations with $^{14}$C-2-deoxyglucose autoradiographic technique$^{12}$ have implicated the involvement of the anterior hippocampal formation and the amygdaloid complex in the pathogenesis of most complex partial seizures in a variety of experimental models of epilepsy$^{12,15,18}$.

Studies of regional glucose metabolism in both
focal epilepsy and generalised epilepsy have all indicated marked differences in the involvement of different brain regions. A study of SE induced by PTZ (pentylenetetrazol) demonstrated that after 1, 5, 15, 20, 60, and 90 min of seizure activity (120-150 mg/kg, intrarterially) marked increases occur in certain areas. These included, hippocampus, most areas of the cerebral cortex, the striatum and the reticular formation of the brainstem. Furthermore, the pattern of activation, once established, tended to persist while the seizure lasted. The striking exception was the substantia nigra. This nucleus showed initially a marked increase in glucose use after 1, 5 and 15 min of seizure activity and a marked lack of activity 90-min after seizure induction.

The anatomic substrates activated during seizure activity differ from those activated during interictal intervals. We have recently showed that increased metabolic rate in the lateral posterior thalamic nuclei during the interictal period may be a result of the activation of cerebral circuits controlling SRSs initiation and/or generalisation.

The patterns of 14C-2-deoxyglucose uptake associated with the less severe forms of status activity primarily involve parts of the amygdaloid complex and the ventral hippocampal formation, while the more severe, convulsive levels also involve widespread structures throughout the ventral forebrain. White and Prince have characterised the neural substrates of both subconvulsive and convulsive forms of SE. The major finding is that persistent focal seizure activity induced by the electrical stimulation of the amygdala or the olfactory cortex leads to the development of one of four discrete levels of self-sustaining SE. Each of these is consistently associated with the activation of distinct anatomical structures. They showed that the amygdalo-hippocampal area is the focus of the most restrictive form of limbic status, type I. The other, regarding more expansive forms of types II and III status, also involve the amygdalo-hippocampal area, but engage more widely distributed structures throughout the ventral forebrain. The anatomical relationships among these structures suggest that the basal nucleus of amygdala and the ventral hippocampal formation should be important for the generation and expression of these more widespread seizure states. Their subsequent experiments were designed to identify the source or sources of the epileptiform activity in type II and III status, with special emphasis on the roles of the basal nucleus and the ventral hippocampal formation. The major findings are that the basal nucleus of the amygdala is the primary epileptogenic focus of both seizure states, and that the ventral hippocampus is additionally involved in the development of sustained ictal discharges with facial and forelimb clonus.

On the other hand, the increased metabolic rate in the caudate putamen (striatum) may be a result of neuronal circuits involved in seizure control. The caudate putamen represents the largest receiving area of the basal ganglia. This region transforms motor information coming from the cortex and conveys it to output nuclei, e.g., the substantia nigra, the entopeduncular nucleus and the globus pallidus.

The increase in glucose metabolism within the substantia nigra suggests that take part in an important circuit for the initiation and propagation of seizure activity within the limbic system. Alternatively, pathways interconnecting substantia nigra with the limbic forebrain are responsible for its modulator effect on the limbic seizure threshold. They may diffusely innervate almost all parts of the limbic system and thus render it capable of controlling the neuronal activity and regulating the neuronal excitability throughout the brain. Surprisingly, 14C-2DG autoradiographic monitoring of limbic seizures in rats produced by focal application of picrotoxin or penicillin into the entorhinal cortex shows that the substantia nigra becomes activated relatively late in the course of seizures, making the first proposal rather unlikely. In fact, autoradiographic studies on the functional anatomy of limbic seizures disclosed that amygdala acts as an exit for propagation of limbic seizures to extrapiramidal pathways, where the paroxysmal activity can be relayed forward resulting in emergence of motor phenomena.

The pre-tectal area (PT) is interconnected with superior colliculus and zona incerta. These structures receive projections from the cortex and have widespread projections that might influence seizure activity. In the other hand, since the activation of this structure (PT) also correlated with maximal convulsive activity, it is possible that PT play a role in seizure motor expression, in view of their numerous descending projections to cerebellum.

The importance of the thalamus in epileptic syndromes obviously correlates with its extensive projection to cortical and other areas. Thalamic areas are also able to reduce epileptiform activity if they were stimulated electrically. In humans, electrical stimulation of the centromedial, anterior or reticular nuclei reduced different types of epilepsy.
In summary, our results suggest that these differences may be particularly important for understanding triggering and spreading mechanisms underlying epileptic activity during status epilepticus and recurrent seizures.

REFERENCES