Temporal and spatial changes in synaptic vesicle glycoprotein 2A (SV2A) under kainic acid induced epileptogenesis: An autoradiographic study

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\textbf{A R T I C L E   I N F O}

Keywords:
- \(^{[3]}\text{H}\)UCB-J
- SV2A
- Epileptogenesis

\textbf{A B S T R A C T}

Synaptic Vesicle Glycoprotein 2A (SV2A) has been proposed as a presynaptic marker in several neurological disorders. Not only is SV2A the target for the antiepileptic drug levetiracetam, but also considered a marker of mature pre-synapses. In this study, we aimed to assess the binding of \(^{[3]}\text{H}\)UCB-J as a selective radioligand for SV2A to visualize and determine changes during different stages of epileptogenesis in vitro autoradiography in rat models of temporal lobe epilepsy. Two different kainic acid (KA) injection routes were used to model temporal lobe epilepsy in the rat; a systemic (10 mg/kg KA injected intraperitoneally) and a local model (1.875 mM KA injected intrahippocampally). Brain tissue was sampled at different time points after the initial status epilepticus and semi-quantitative \(^{[3]}\text{H}\)UCB-J autoradiography was performed to determine temporal and spatial changes under the progression of epileptogenesis. A decrease in \(^{[3]}\text{H}\)UCB-J binding was observed in many brain areas in the acute phases after both types of kainic acid administration. Peak reductions occurred slightly before in systemic-treated animals (within 3–10 days) than after local-treated animals (within 5–15 days). Interestingly in the systemic model, we observed a full restoration in the binding level 30 days after the treatment in most areas probably reflecting neuronal reorganization. However, after the local injection in the hippocampus, the binding in the hippocampus, and in temporal and perirhinal cortices did not return to basal levels. The time-course profile displayed lateralization in the local model. These results demonstrate changes in the amount of a presynaptic SV2A binding site after seizures and suggest that SV2A may have importance in eliciting spontaneous seizures and/or be a biomarker for epileptogenesis. The present study shows that SV2A is a biomarker of acute phase epileptogenesis in specific brain regions.

1. Introduction

Temporal lobe epilepsy (TLE) is a common neurological disorder characterized by the occurrence of recurrent spontaneous seizures that arise from an imbalance between excitation and inhibition networks in the temporal lobe (Avoli et al., 2016; Salami et al., 2020). There are no preventative and curative treatments for TLE, as anti-epileptic drugs provide only symptomatic treatment of seizures. Approximately one-third of patients are more or less resistant to therapeutic agents (Lösch et al., 2020). It is still unknown why some patients develop treatment resistance, though reorganization and plastic changes of the brain are considered to play a role (Beck and Yaari, 2012).

Epileptogenesis is the process in which a normally functioning brain is transformed into a brain that supports unprovoked recurrent seizures (Pan et al., 2018). This transformational process includes various biological changes such as neuronal death, synaptic loss, and abnormal reorganization of synapses resulting from the axonal and dendritic sprouting (Pan et al., 2018; Pitkanen et al., 2015). Such cellular alterations eventually lead to both circuit-level changes such as GABAergic interneuron loss and generation of recurrent excitatory synaptic connections (Botterill et al., 2019) and anatomical alterations such as hippocampal atrophy (Finegersh et al., 2011).

Synaptic vesicle glycoprotein 2A (SV2A) is a presynaptic vesicular protein and mice with SV2A deletion displays convulsions (Crowder
et al., 1999). Furthermore, SV2A is the target of antiepileptic drugs, levetiracetam (Lynch et al., 2004) and brivaracetam (Rogawski, 2016). Clinical studies also support that SV2A protein levels are decreased in the hippocampus and cortex in TLE patients (Crévecoeur et al., 2013; Feng et al., 2009; Van Vliet et al., 2009). All these data strongly indicate that SV2A is involved in the underlying mechanism in epilepsy even though the molecular and cellular function(s) of the protein remains to be established.

More recently, SV2A has been suggested as a synaptic density biomarker (Becker et al., 2020; Cai et al., 2019; Heurling et al., 2019). SV2A radioligands used in imaging studies of epilepsy patients displayed lower binding in several brain areas as a reflection of synaptic loss (Finnema et al., 2020). Although it has been shown that SV2A decrease may contribute to the progression of epilepsy in rats (Van Vliet et al., 2009; Contreras-García et al. (2018), little is still known about the detailed time-course changes of SV2A level during epileptogenesis. Thus, detailed monitoring of SV2A during this transformational process of the brain into an ‘epileptic brain’ may provide a valuable approach to trace stages of synaptic reorganization in TLE.

Since the detailed evaluation of the epileptogenesis process in the human brain is impractical due to the limitation caused by evaluated time points, preclinical studies with well-validated animal models are required to elaborate on the entire process. Kainic acid (KA) is used as a neurotoxin that induces rapid neuronal degeneration of GABAAergic interneurons in the hippocampus (Lévesque and Avoli, 2013) and thereby causes seizures. Both systemic and local KA treatments are commonly used as animal models of TLE (Bertoglio et al., 2017; Lévesque and Avoli, 2016, 2013).

Changes of SV2A have been demonstrated with in-vivo imaging by [18F]UCB-J tracer in systemic KA model (Serrano et al., 2020), but the detailed temporal profile was not ruled out. Also, the resolution was limited tempting us to examine changes in binding by high-resolution autoradiography in two rat models several months after the treatment. Furthermore, any differences between systemic and local KA administration have not been investigated. In this study, we used [3H]UCB-J, a validated radiotracer for in-vitro autoradiography with high affinity to SV2A, as a tool to determine changes in binding during key stages of epileptogenesis in both systemic and local KA treatment models of TLE. For that purpose, we constructed two independent time-course experiments by sampling brain tissue at 8 different time points representing acute (1–3–5 post day status epilepticus) (pSE), latent (10–15–30 post day), and chronic phases (60–90 post day) of epileptogenesis (Goldberg and Coulter, 2013).

2. Methods

2.1. Animals and ethics

The protocol for the study has received approval by the Institutional Animal Care and Use Committee and all studies were conducted in accordance with the United States Public Health Service’s Policy on Humane Care and Use of Laboratory Animals (Approval no. EGEHA-YMER-2019-003).

2.2. Experimental design I - systemic KA treatment

In order to establish the systemic KA model, we injected Sprague-Dawley adult male rats (200–250 g) with 10 mg/kg KA intraperitoneally (i.p.). To construct a time-course study, we divided the animals randomly into 8 different groups according to brain sampling time points (1, 3, 5, 10, 15, 30, 60, and 90 days after status epilepticus). The age-matched control groups for every sampling point (n = 6) were constructed by i.p. sterile saline solution injection. The animals were sacrificed by cervical dislocation under ketamine (75–100 mg/kg i.p.)- xylazine (10 mg/kg i.p.) anesthesia. The brains rapidly removed from the skull, and kept at – 80 °C until further processing.

2.3. Experimental design II - local KA treatment

Under ketamine (75–100 mg/kg i.p.) - xylazine (10 mg/kg i.p.) anesthesia, animals were placed onto the stereotaxic frame. Bregma and lambda reference points were set for every animal. Anteroposterior (AP) – 2.04 mm; Mediolateral (ML) 1.10 mm; Dorsoventral (DV) 3.81 mm coordinates were determined for dorsal hippocampus injection by using a rat brain atlas (Paxinos and Watson, 2007). Animals were injected with 0.8 μg/2 μl (1.875 mM) KA dissolved in sterile saline using a stereotaxic robot (Neurostar GmbH, Germany) with a 1.0-μl Hamilton microsyringe (30 G cannula and a flow rate of 1 μl/min). Repulse of the injected solution was prevented by waiting for 5 min in the injection area. Pre- and post-surgical care of animals were performed according to instructions described previously (Fornari et al., 2012). Animals were grouped, sacrificed, and the brains were sampled as described above for the systemic design. Age-matched control groups (n = 6) were sham-operated by sterile saline injection using the same stereotaxic procedure and sacrificed during the same experimental time schedule (1, 3, 5, 10, 15, 30, 60, and 90 days after first status epilepticus).

2.4. Behavioral evaluation

The convulsive behaviors of the KA-treated rats were scored based on the scale of 0–6 modified from Racine’s Scale (Racine, 1972). Rats were video-monitored for 10 h during the light period during 90 days. Rats displaying at least 4th degree seizure within 4 h after KA treatment were included in all tested time points. In addition to showing at least 4th-degree seizure, the rats exhibiting spontaneous recurrent seizures (SRs) within a month were used for 30, 60 and, 90 post dose. Only the rats that fulfilled the criteria defined were included in the longitudinal autoradiography study which allowed 6 animals/per group in both models.

2.5. In-vitro autoradiography and image analysis

20 μm-thick serial coronal cryostat sections from each animal (n = 6) were mounted on Super frost glass slides (Thermo Scientific). The sections were selected along the rostro-caudal axis according to different distances to the bregma and content of regions of interest (ROI). The mounted forebrain sections were collected at the level of i) the prefrontal cortex and nucleus accumbens [- 3.2 mm to bregma], ii) the main portion of the striatum [+ 1.2 mm to bregma], iii) the hypothalamus and dorsal hippocampus [- 3.6 mm to bregma], and iv) the ventral hippocampus [- 5.2 mm to bregma]. To reduce possible variations between the tissue, experiments were performed on sections adjacent to each other. At least 3 technical replicates (sections/slide) were used. The sections were pre-incubated twice for 10 min at room temperature in a 50 mM Tris-HCl buffer (pH 7.4) containing 0.5% bovine serum albumin (BSA), 5 mM MgCl2, 2 mM EGTA. Incubation was performed for 60 min in a preincubation buffer containing 6 nM of [3H] UCB-J (generously supplied by UCB Pharma, Belgium). Subsequently, slides were washed twice in an ice-cold preincubation buffer for 10 min and briefly dipped in an ice-cold distilled water before being dried overnight in a paraformaldehyde chamber. Glass slides were then exposed to FUJI imaging phosphor plates for three days at 4 °C together with [3H] standard ARC (American Radiolabeled Chemicals, Inc, USA) and [3H] microscale Batch 21 A (GE Healthcare, UK).

The FUJI imaging plates were scanned by FujiFilm Image Reader ( BAS-2500 V1.8) and autoradiograms were analyzed with Image J software (Version 2.0.0, NIH). The ROIs were delineated by using rat brain atlas (Paxinos and Watson, 2007), and measurements were done from 3 individual sections per animal. The white matter value of each section was subtracted from the values of ROI measurements in the same tissue sections. This calculated grey value optical densities were converted to the standards of known concentrations. The interpolated values were then calculated into the amount of bound radioligand [fmol/mg Tissue Equivalent] in the tissue,

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and the mean of these values for the different sections was used for each animal.

2.6. Statistical analysis

GraphPad Prism (version 8.02) was used to perform statistical analysis. One-way analysis of variance (ANOVA) followed by post-hoc Tukey test was performed for multiple comparisons of $[^3]$H$[^3]$UCB-J binding levels between the sole control group (which represents the average of every age-matched controls) and KA-treated groups. Student $t$-test was performed to compare differences between ipsilateral and contralateral brain regions to the injection site. While the ANOVA test is used for comparison of % reductions among brain regions (such as % reductions in hippocampus vs cortex); Student $t$-test was performed for comparison of % reductions of binding in ventral and dorsal parts of the regions. In this study $P$-values less than 0.05 were considered significant.

3. Results

3.1. Behavioral scores of KA-treated rats

While rats administered systemic KA experienced their first status epilepticus $42 \pm 17$ min after injections, it was observed $116 \pm 47$ min after injection in rats administered local KA. During the first 4 h after injections, convulsions were rated and the following behavioral stages were observed: (1) sudden behavioral arrest or immobility, (2) orofacial movements (3) neck jerks and wet-dog head shakes, (4) unilateral or bilateral forelimb clonus, (5) tonic-clonic seizures with the animal falling accompanied by exophthalmos and salivation, (6) wild jumping, and tonic extension of either forelimb leading to death. The time to onset of manifestation of first SRSs was diverse among animals as noted from daily video recordings for both models. After SE induction with KA, SRSs occurred between 14 and 88 days later. SRSs were provoked within a similar time scale for both models.

3.2. $[^3]$H$[^3]$UCB-J binding in systemic KA model

The binding of $[^3]$H$[^3]$UCB-J was observed throughout the grey matter.

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**Fig. 1.** Representative autoradiogram images of animals exposed to systemic injections of kainic acid at the level of the dorsal hippocampus (sections = 3.6 mm distance to bregma). Only autoradiograms of the dorsal portions are presented (A), while the quantitative graphs (B-G) show both the dorsal and ventral portions. Delineations of the subregions such as CA1, CA3 and the dentate gyrus were defined according to the anatomical borders defined in rat brain atlas (Paxinos and Watson, 2007) for dorsal (-3.6 mm distance to bregma) and ventral (-5.2 mm to bregma) hippocampus. Quantitative analysis of the level of binding in the dorsal CA1 (B), CA3 (D), dentate gyrus, granular layer dorsal (F), and ventral CA1 (C), CA3 (E), dentate gyrus, granular layer ventral (G) subparts of the hippocampus. The asterisk represents the significant difference compared to control according to the One-way ANOVA test. Control is the mean value of all age-matched controls. n = 6 for all groups.
regions. However, some regions displayed more labeling than others (Figs. 1 and 2). By contrast, white matter displayed virtually no binding. One of the most densely labeled regions was the hippocampus. Particularly the areas with densely packed cell layers such as cornu ammonis and granular cell layer showed larger binding levels presumably reflecting the high cell and synapse density. We analyzed the level of binding in subregions of the dorsal and ventral hippocampus, in several cortical and subcortical regions, as well as in white matter. Representative images showed different binding profiles during acute, latent, and chronic phases. Notably, no differences after KA treatment was observed in the white matter. When comparing the two hemispheres, no lateralization was found in any region (data not shown).

3.2.1. Hippocampus

The binding level was analyzed in subregions of the dorsal and ventral hippocampus. There was a gradual decrease in the binding in the hippocampus during acute phases of epileptogenesis; particularly the lowest level was found between 3 and 10 days. At time points after 3–10 days, the binding profile showed a time-dependent increment. These time points represent the latent and chronic phases of $[^3H]$-UCB-J binding level. Importantly, the dentate gyrus showed larger and more permanent reductions than other subregions (Fig. 1A).

In all regions examined, the reduction in binding was largest at 5 days ($p < 0.0001$ compared to baseline). At this time point, the level of binding was 38%, 37%, 63% lower compared to control in dCA1, dCA3, dDG (Figs. 1B, 1D, 1F). The reduction was smaller in ventral parts than dorsal parts in CA3 ($p < 0.05$) and DG regions ($p < 0.001$), where the level of binding was 29%, 34%, 51% lower compared to control in vCA1, vCA3, vDG, subsequently (Fig. 1C, 1E, 1G). At 30 days, the binding levels in both the dorsal and ventral CA1 and CA3 was not statistically different from baseline. In contrast to the other hippocampal subregions, a major reduction was observed in the dDG after 30 days (Fig. 1F). At 90 days after systemic KA, no difference was observed compared to control in any part of hippocampus.

3.2.2. Cerebral cortex

Strong radioligand binding was observed throughout the cerebral cortex. Time profile of changes in binding in different cortical regions such as prefrontal cortex (Fig. 2A), orbitofrontal cortex (Fig. 2B), cingulate cortex (Fig. 2C), and sensorimotor cortex (Fig. 2D) was similar between these regions and to the hippocampus. As for the hippocampus, and in all parts of the cortex, the largest reductions were detected at 5 days ($p < 0.0001$) (Fig. 2A-2D). Compared to baseline control, reductions in binding at this time point was detected to be 18%, 26%, 25%, and 15% for the cingulate, sensorimotor, prefrontal, and orbitofrontal cortex, respectively.

After the 5 days time-point, the level of binding gradually increased in all neocortical areas examined. In the cingulate cortex, the level of binding was not statistically different from baseline controls 15 days after the treatment. For the other regions, such as the orbitofrontal, prefrontal, and sensorimotor cortices, the level of binding continued to be significantly lower until 60 days after treatment. A similar time profile in binding as for the cingulate cortex was observed in the

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**Fig. 2.** Quantitative analysis of different cortical areas in systemic KA-treated animals. Prefrontal cortex [sections + 3.2 mm distance to bregma] (A), orbitofrontal cortex [sections + 3.2 mm distance to bregma] (B), cingulate cortex [sections + 3.2 mm distance to bregma] (C), sensorimotor cortex [sections + 1.2 mm distance to bregma] (D). The asterisk represents the significant differences compared to control according to One-way ANOVA test. Control represents mean value of all age-matched controls. $n = 6$ for all groups.
temporal and piriform cortices (data not shown).

### 3.2.3. Subcortical areas

The time profile in binding in the subcortical areas was different from both the cortex and hippocampus. In the caudate-putamen, a rapid decline was seen after 1 day ($p < 0.01$) (Fig. 3B). In the nucleus accumbens, a strong reduction in binding was detected after 3 days ($p < 0.0001$) (Fig. 3C). In the striatum, the lowest level of binding was among the time point examined seen after 10 days (43% and 50%, subsequently) ($p < 0.0001$). The level of binding was in all subcortical areas was after 60 days not different from basal levels (Fig. 3A-3B).

By contrast, virtually no change in $[^{3}H]$UCB-J binding was observed in the hypothalamus at all-time points examined (Fig. 3D). Importantly, no difference in binding was observed at any time point in the corpus callosum (Fig. 3E).

#### 3.3. $[^{3}H]$UCB-J binding in local KA model

Intrahippocampal injections of KA in the right dorsal hippocampus caused changes in $[^{3}H]$UCB-J binding both in the hippocampus and in extra-hippocampal regions, and time profiles show similarities to the systemic KA model. In the local treatment, there was a rapid structural damage and dispersion of the cellular layers of the hippocampus at 1 and 3 days after local KA treatment. In representative images, scattered labeling displays qualitative loss in cellular layers, particularly in the DG (Fig. 4A). Also, certain areas in the temporal cortex showed a heterogeneous binding pattern. Relative lower levels of binding, likely due to cell loss, was seen in and around of the injection site in the early phases. Apart from the immediate changes around the site of the injection, there was a lateralization in the level of binding in the acute phases between the two hemispheres. The lateralization in binding profile was seen in and around the injection site, and in the surrounding tissues involving not only the hippocampus, but also the temporal and piriform cortices. Notably, the lateralization was not detected in any region in the chronic phases (at day 10 and after) of epileptogenesis.

Further, as mentioned under the experimental methods, we tested the effect of the intrahippocampal injection itself, which may lesion tissue, by conducting age-matched sham-operated control groups. When we compared ROIs (such as cortex and hippocampus) in naive rats sacrificed directly without any intervention ($n = 6$) as well as in sham-operated rats ($n = 6$ per group; injected intrahippocampally with saline) and subsequently sacrificed on days 1, 3, 5, 10, 15, 30, 60, and 90; no prominent difference in terms of binding density was observed. Therefore, the control group represents the mean binding level of all sham-treated control groups.

#### 3.3.1. Hippocampus

A notable decline in binding was seen in both dorsal (Fig. 4, B-D-F) and ventral subregions (Fig. 4, C-E-G). Due to the lateralization, the decrease of the binding in the ipsilateral site was larger at earlier time points, though significant in both hemispheres.

A prominent reduction in binding was observed after 1 day in all ipsilateral subregions of both dorsal and ventral hippocampus. On the contralateral site, the decline in binding was apparent after 3 days ($p < 0.05$). Even though there were regional differences, the magnitude in the reduction was prominent after 5 days, but peaked after 10 days in all ROIs. The decreases were about 45% in dCA1 (Fig. 4B), 56% in vCA1 (Fig. 4C), 52% in dCA3 (Fig. 4D), 51% in vCA3 (Fig. 4E), 62% in dDG (Fig. 4F), and 52% in vDG (Fig. 4G). As described above, differences in the binding level between the ipsilateral and contralateral sites were observed after 1 day, 3 days, and 5 days (acute phases). At the time point where the binding in the hippocampus was at the lowest level around 10 days no difference in binding was seen between the two sites. The binding level increased slightly until 90 days. In contrast to the time profile seen after systemic treatment with KA, the binding level never reached control levels in any of the hippocampal regions after intrahippocampal KA.

#### 3.3.2. Cortex

The time profiles in binding seen in cortical subregions were quite
different from each other and from the profiles observed after systemic administration of KA. In the prefrontal cortex (Fig. 5 A), virtually no change in binding was seen at any time point. In the sensorimotor cortex (Fig. 5 B), the binding level declined in the first days, and a reduction of 30% was detected after 3 days. However, no lateralization was found after 30 days (Fig. 5 B).

By contrast, in the temporal (Fig. 5 C) and piriform cortices (Fig. 5 D), pronounced reductions and lateralization were seen after only 1 day (42–48% in temporal and 40–49% in piriform cortex). As for the hippocampus, the lowest level of binding was seen after 10 days. At later time points, the level of binding showed no difference between the two hemispheres, but binding level remained lower until 90 days after the treatment in both cortical areas at both sides (Fig. 5 C and 5D).

3.3.3. Subcortical areas
The temporal binding profile in binding in selected subcortical areas was also region specific. For example, levels of binding in the caudate-putamen (Fig. 6A-C) resembled the profile of the sensorimotor cortex and displayed a strong reduction after 3 days, and the level returned gradually to basal levels over time. The time profiles between the
caudate-putamen (Fig. 6B) and the nucleus accumbens (Fig. 6C) was similar. In the hypothalamus, the binding levels over time was constant, with only a minor but notable difference at a single time point (Fig. 6D). In the corpus callosum, no difference was seen at any time bilaterally (Fig. 6E), as also observed in other white matter regions examined at other levels of the brain (data not shown).

4. Discussion

This study shows that both systemic and local KA administration produce reductions in the binding of \(^{3}H\)UCB-J in both hippocampal and extra-hippocampal grey matter areas in the first days after treatment with KA. The demonstration of rather similar changes in two independent animal experimental models add to the reproducibility of the findings. The magnitude differs between regions and between the two KA models. Importantly, and as discussed below the reduction in the binding is followed by a complete restoration in most brain areas.

It was evident and expected that the hippocampus was the mostly affected region. This agrees with the fact that the hippocampus is the most vulnerable region to KA neurotoxicity and KA receptors are more abundant than in any other region (Vincent and Mulle, 2009). Early damage takes place in the hippocampus also supports this region to be the site of seizure onset (Vincent and Mulle, 2009; Zhu et al., 2011). \(^{3}H\) UCB-J binding in white matter is negligible and is not affected by KA treatment regardless of the administration route, which would be expected due to the lack of synapses (Rossano et al., 2020). Accordingly, the white matter has previously been used as a reference region for SV2A radioligands in imaging (Finnema et al., 2016; Rossano et al., 2020). Virtually no change in binding was observed in the thalamus emphasizing a region-specific regulation.

The KA model is a translational model of TLE, which represents the epileptogenesis process with all aspects including degenerative and neuroplastic changes in the cerebral cortex and hippocampus (Lévesque and Avoli, 2016; Isaeva et al., 2013; Tzeng et al., 2013; Cavazos and Cross, 2006; Suárez et al., 2012). The time profile of \(^{3}H\)UCB-J binding in the hippocampus shows a gradual decline in early phases and a progressive restoration in the later phases. The reduction in the binding could be either cell death, synaptic loss, or changes in vesicle numbers or binding affinity. Elevation in binding might be a result of synaptic reorganization which often takes place during latent and chronic periods of epileptogenesis and endures for months or years in mammals (Botterill et al., 2019).

Many of the plastic changes cause imbalance between excitatory and inhibitory synapses and lead to abnormal synaptic plasticity and gliosis particularly in hippocampal structures (Goldberg and Coulter, 2013; Pan et al., 2018; Puttachary et al., 2015). Because of this abnormal reorganization in latent phases, patients will experience spontaneous recurrent seizures, which is the hallmark symptom of epilepsy (Gu and Dalton, 2017). Given that the level of radioligand binding indicates synaptic density (Becker et al., 2020), our data demonstrates synaptic density monitored by \(^{3}H\)UCB-J binding returns back to baseline. However, whether this density is manifested as functional synapses is uncertain and remains to be established.

We suggest that the recovery in synapse density reflected by \(^{3}H\) UCB-J binding may not necessarily be a reversible recovery, but may represent changes in synapses that shift towards more excitable networks in the hippocampus. A previous in-vivo imaging study with \(^{18}F\) fluordeoxyglucose ligand demonstrated time-dependent metabolic changes of the brain in epileptic rats (Guo et al., 2009). Similar to the present findings, brains from rats with status epilepticus showed a gradual reduction in brain glucose metabolism in acute and latent phases of epileptogenesis. Strikingly, glucose hypo-metabolism was
persistent in the hippocampus in latent and chronic phases similar to the reduction in $[^3H]$UCB-J binding after local KA injection. The overlap in the time profile of binding and glucose metabolism could indicate a relationship between synaptic loss and accompanying hypometabolism at the early phase and during later phases returned into synaptic reorganization and relatively increased metabolism.

Another aim in applying two different treatment routes was to investigate possible changes in the time profile of $[^3H]$UCB-J binding within ipsi- and contra-lateral sites as we believe such analyses have not been ruled out before. We know that while systemic injections cause whole-brain excitotoxicity, local intracerebral injections cause local excitotoxicity (Levesque and Avoli, 2013; Mohd Sairazi et al., 2015). The present results demonstrate that the reduction in binding occurred earlier after local administration, which likely is a result acute neuronal loss (Jin et al., 2009). Thus, intrahippocampal KA induces acute necrotic death, while in systemic treatment, cells be lost due to programmed cell death at later time points (Liu et al., 2012). On the other hand, maximal reduction was seen 10 days after local, and 5 days after systemic treatment. The antagonistic effect of ketamine that was used for anesthesia in the local treatment model may contribute to these time differences (Zorumski et al., 2016; Vermoesen et al., 2010).

Previous studies showed that intracerebral KA injections in the hippocampus induce cellular loss that occurs within 48 h on the injected site, but later in the contralateral site via spread of ictal activity (Li et al., 2018). The seizure ictal activity can be propagated from dorsal to ventral parts of the hippocampus and to extrahippocampal and contra-lateral sites by axonal projections (Ben-Ari et al., 2008; Li et al., 2018; Zeidler et al., 2018). The propagation of abnormal electrical activity may cause changes in synaptic density within 5 days (Ben-Ari et al., 2008; Li et al., 2018). Consistently, immediate reductions in binding in
the hippocampus, temporal cortex, and piriform cortex ipsilateral to the injection within the first 3 days is followed by a delayed reduction in the contralateral site. This lateralization was limited to the acute phases up to 10 days after treatment and disappeared later.

In the systemic model, we revealed that reduction in binding in the hippocampus and throughout the cerebral cortex returned to basal levels with full recovery months after administration. By contrast, local KA injections lead to large reductions in the hippocampus, temporal and piriform cortex, but these levels did not return to basal levels, even 3 months after treatment. We propose that local KA treatment causes more drastic changes and different outcomes in circuits that are not manifested in behavioral symptoms. Notably, EEG analysis performed 3 months after SE revealed no correlation between any EEG parameter evaluated and [F18]UCB-H uptake in the brain (Serrano et al., 2020).

Interestingly, extra-hippocampal areas including cortical and subcortical regions are also affected. The prefrontal cortex, orbitalfrontal cortex, cingulate cortex, and sensorimotor cortex showed milder reductions when compared to the hippocampus in both treatment models. However, the magnitude of reductions was higher in the temporal and piriform cortices. Previous studies revealed that KA induces pronounced lesions in the piriform cortex (Sprengers et al., 2014) and temporal cortex (Zhu et al., 2011; Kodanakadath et al., 2021). However, also the prefrontal, cingulate, and orbitalfrontal cortices are influenced by KA toxicity (Gill et al., 2017; Bartolomei et al., 2008). Additionally, subcortical regions such as caudate-putamen and accumbens also displayed significant reductions in binding. It is known that KA causes striatal lesions (Coyle and Schwarz, 1976). The reduction of binding outside the hippocampus is likely attributed to ictal activity but not KA itself, impacting multiple brain networks (Carboni et al., 2020).

Reductions in SV2A mRNA level, protein expression, and specific radioligand binding density have been reported in the hippocampus and the neocortex of TLE patients (Grèceveur et al., 2014; Feng et al., 2009, 2020), and the SV2A levels were shown to be correlated with the degree of hippocampal sclerosis in TLE patients (Grèceveur et al., 2014). These studies provide important knowledge about changes of SV2A level in the brain of TLE patients and corresponds better with the local KA as the changes are likely permanent.

Given SV2A is a reliable marker of synaptic density, the data here suggest that determination of [H]UCB-J binding by autoradiography is a valuable approach to measure changes in disease stage-, and brain region variability changes, or reorganization of neuronal circuits, not only in epileptogenesis but also in other neurodegenerative disorders.

Funding

This study was funded by the scientific research council of Izmir Katip Celebi University (Grant no: 2019-TDR-SABE-0002). BAP is funded as a PhD student by the Scientific and Technological Research Council of Turkey under 2214 scholarship program during this study. JDM is funded by the Elsas and the NOVO Nordisk Foundations.

CRediT authorship contribution statement

JDM, BAP, and EOO conceived the idea and planned the project BAP and CBE conducted animal experiments. BAP and CAM carried out the autoradiography and performed the data analysis. JDM and BAP wrote the manuscript, and all authors contributed to the final version of the manuscript.

Conflict of Interest

All authors have reported no relevant conflict of interest.

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