RESEARCH ARTICLE

Effects of Valproate on Cerebral Amino Acid Neurotransmitters during K^+- Evoked Cortical Spreading Depression in Rats

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Abstract

This study aimed to investigate the effects of valproate on the levels of cerebral amino acid neurotransmitters during K^+- evoked cortical spreading depression (CSD) in male Wistar rats (200-300 g) using microdialysis technique. The rats were anesthetized by urethane (1.5 g/kg, i.p.) and then placed in stereotaxic frame. The scalps were cut and opened to expose the skulls and two burr holes were drilled on the right hemisphere. The anterior hole at the frontal bone was to place microdialysis probe (aCSF, 2 µl/min flow rate), whereas the posterior hole at the parietal bone was used for an application of solid KCl (3 mg). Microdialysis probe was inserted into frontoparietal cortex and intraperitoneal injection of either 0.5% Carboxymethyl methyl cellulose sodium (1 ml/kg) or valproate (200 mg/kg), was made following a stabilization period of 60 minutes. Thirty minutes after administration of the test compounds, CSD was induced by placing solid KCl onto the brain and observation was made for another 90 minutes. Dialysates were collected every 30 minutes throughout the experimental period and being analyzed for amino acid neurotransmitters by High Performance Liquid Chromatography with Fluorescent Detection. It was found that levels of both cortical excitatory (glutamate and aspartate) and inhibitory (GABA and glycine) amino acid neurotransmitters in VPA-treated group were significantly decreased in comparison to those of CMC-treated group at any observed times (p = 0.05). However, the depression was greatest on glutamate which is a major excitatory amino acid neurotransmitter whereas smaller effect was noted on the others. The results obtained from this study were consistent with electrophysiological depression of CSD by VPA previously reported by other investigators and may explain the effect of VPA in migraine prophylaxis.

Key Words Microdialysis, Cortical spreading depression, Valproate, Amino Acid Neurotransmitters

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ผลของวาลโปรเอทต่อระดับสารสื่อประสาทบริเวณซีรีบรัล คอร์เท็กซ์ที่ถูกกระตุ้นให้เกิดคอร์ติคัลสเปรดดิง ดีเพรสชันในหนูแรท

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บทคัดย่อ

การวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาผลของวาลโปรเอท ต่อการเปลี่ยนแปลงของระดับสารสื่อประสาทที่เป็นกรดอะมิโนบริเวณซีรีบรัล คอร์เท็กซ์ ในขณะกระตุ้นให้เกิดปรากฏการณ์คอร์ติคัลสเปรดดิง ดีเพรสชันในหนูแรทเพศผู้ ขนาดน้ำหนักตัว 200-300 กรัม โดยใช้วิธีไมโครไดอะลัยซิส ทำการสลบหนูแรทด้วยยูรีเทรนขนาด 1.5 ก/กก หลังจากยึดศีรษะหนูแรทไว้กับ stereotaxic frame เรียบร้อยแล้ว เปิดผิวหนังที่บริเวณศีรษะของหนูแรท ใช้เครื่องกรอฟัน เจาะรู 2 รู บริเวณกระโหลกศีรษะ โดยที่รูด้านหน้าใช้ในการเก็บสารสื่อประสาทที่ซีรีบรัล คอร์เท็กซ์ และรูด้านหลังใช้สำหรับวางผลึกโพแทสเซียมคลอไรด์ขนาด 3 มิลลิกรัม หลังจากนั้นฝัง microdialysis probe ที่บริเวณรูด้านหน้า แล้วบันทึกค่าปกติเป็นเวลา 60 นาที ก่อนให้สารทดสอบ คือ คาร์บอกซีเมทิลเซลลูโลสขนาด 1 มล/กก และวาลโปรเอท ขนาด 200 มก/กก ซึ่งให้โดยการฉีดเข้าทางช่องท้อง จากนั้นกระตุ้นให้เกิดปรากฏการณ์คอร์ติคัลสเปรดดิง ดีเพรสชันโดยการวางผลึกโพแทสเซียมคลอไรด์ แล้วเก็บตัวอย่างเป็นเวลา 90 นาที โดยเก็บทุก ๆ 30 นาที ตัวอย่างที่เก็บได้นำไปวิเคราะห์หาปริมาณสารสื่อประสาท ด้วยเครื่อง High Performance Liquid Chromatography with Fluorescent Detection (HPLC-FLD) ในการศึกษานี้พบว่าวาลโปรเอท ขนาด 200 มก/กก มีผลทำให้ระดับสารสื่อประสาททั้งชนิดกระตุ้น (กลูตาเมทและแอสพาเตท) และยับยั้ง (กลัยซีนและกาบา) ลดลงอย่างมีนัยสำคัญทางสถิติเมื่อเปรียบเทียบกับกลุ่มที่ได้รับคาร์บอกซีเมทิลเซลลูโลส (p = 0.05) อย่างไรก็ตามวาลโปรเอทมีผลในการลดระดับกลูตาเมทซึ่งเป็นสารสื่อประสาทหลักที่มีฤทธิ์กระตุ้นได้มากที่สุด ในขณะที่มีผลเพียงเล็กน้อยต่อระดับของสารสื่อประสาทชนิดอื่น ๆ สอดคล้องกับผลของวาลโปรเอทในการลดการเกิดคอร์ติคัลสเปรดดิง ดีเพรสชัน ที่รายงานไว้ในการศึกษาด้านสรีรวิทยาไฟฟ้าก่อนหน้านี้ และอาจสามารถอธิบายผลของวาลโปรเอทในการป้องกันโรคไมเกรนได้

คำสำคัญ ไมโครไดอะลัยซิส, คอร์ติคัลสเปรดดิง ดีเพรสชัน, วาลโปรเอท, สารสื่อประสาท

Introduction

Cortical spreading depression (CSD), implicated in the pathogenesis of migraine headaches, is a slowly propagating wave (3-5 mm/min) of neuronal and glial depolarization followed by a long-lasting suppression of neuronal activity (Lauritzen 2001). It can be triggered by several diverse stimuli e.g. direct cortical trauma, lack of energy, electrical or chemical stimulation to the brain including high concentration of K^+ which is, carried out in animals, considered a valid experimental model for the migraine aura (Saito et al. 1995). Suppression of K^+- evoked CSD in animal model has been demonstrated by a number of antiepileptic drugs widely used as prophylaxis of migraine (Ayata et al. 2006, Eikermann-Haerter and Moskowitz 2008, Hoffmann et al. 2010).

Valproate or valproic acid (2-propylpentanoic acid; VPA), a simple branched-chain fatty acid used in the treatment of several seizure types and mania, has been shown in controlled clinical trials to reduce the severity and frequency of spontaneous migraine attacks (Coria et al. 1994, Mathew et al. 1995). Other reports have suggested efficacy in the treatment of chronic daily headache (Mathew and Ali 1991) and cluster headache (Hering and Kuritzky 1989). A variety of mechanism of action of valproic acid may contribute to its effect in migraine treatment. Several study demonstrated that valproate suppressed migraine-related events in the cortex, perivascular parasympathetic nerve or trigeminal nucleus caudalis (Cutrer et al. 1997). There is experimental evidence that it suppresses neurogenic inflammation and directly attenuates nociceptive neurotransmission (Cutrer and Moskowitz 1996). In addition, valproate has been reported to alter levels of excitatory and inhibitory neurotransmitters *in vitro* (Cutrer et al. 1997). Therefore we consider that it is interesting to investigate the effects of valproic acid on cortical amino acid neurotransmitter levels during K^+-evoked CSD in rats.

Materials and Methods

Animals

The experiments were performed in male Wistar rats weighing 200-300 g. The rats were obtained from the National Laboratory Animal Center, Mahidol University, Nakornpathom, Thailand. After arrival the Animal Center of Faculty of Pharmaceutical Sciences, Chulalongkorn University, the rats were housed five rats per cage and kept under controlled environmental condition (ambient temperature 25$\pm$2°C, humidity 60%-70%$\pm$10%, 12-h light/dark cycle). All animals were allowed free access to both standard laboratory chow and tap water and were adapted to housing conditions for at least one week prior to experiments. All animal care and handing were conduced with an approval of the Institutional Animal Care and Use Committee of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

Chemicals

Valproic acid (VPA), and carboxymethyl cellulose (CMC) are chemical reagents used for preparation of artificial cerebrospinal fluid and urethane were purchased from Sigma chemical company.

Preparation of test compounds

VPA was suspended in 0.5% CMC solution.

Experimental protocol

The rats were anesthetized by intraperitoneal injection of 1.5 g/kg of urethane and were maintained under anesthesia throughout the experiments with supplemental injections as needed to suppress blink and hind paw nociceptive reflexes. Two burr holes were made for an implantation of microdialysis probe in one and for an application of KCl. After the surgery, the stabilization period of 60 minutes was allowed before an intraperitoneal injection of 0.5% CMC (1 ml/kg, n=6) or VPA (200 mg/kg, n=6). Thirty minutes after the administration of the test compounds, CSD was induced by the application of solid KCl and the observation was made consecutively for another 90 minutes. Dialysates were collected every 30 min-interval and being analysed for amino acid neurotransmitters namely, glutamate, aspartate, GABA and glycine by High Performance Liquid Chromatography with Fluorescent Detection (HPLC-FLD).

Surgery

After fixing the animal in the stereotaxic frame, a midline incision was made in the scalp from the level of fronto-nasal suture to the neck. The scalp was reflected to expose the skull. Two craniotomies were made by saline-cooled drilling. Anterior craniotomy, used for an implantation of microdialysis probe, was performed in the frontal bone at 1 mm anteriorly and laterally from bregma (7 mm diameter). The posterior craniotomy were performed in the parietal bone at 7 mm posteriorly and 1 mm laterally from bregma (2 mm diameter) for an application of 3 mg solid KCl (Bogdanov 2011).

Microdialysis

After surgery, microdialysis probes (CMA/11, 2 mm membrane length, 0.24 mm diameter, 6 kDa cut-off) were inserted into the frontoparietal cortex (1 mm anterior to bregma, 1 mm lateral to midline, 1.5 mm deep from the cortical surface). The microdialysis probes were perfused with artificial cerebrospinal fluid (aCSF; 120 mM NaCl, 15 mM $NaHCO_3$, 5 mM KCl, 1.5 mM $CaCl_2$, 1 mM $MgSO_4$ and 6 mM glucose, pH 7.4) at a flow rate of 2 μl/min using a microinfusion pumps. Dialysates were collected every 30 min-interval and being analyzed for amino acid neurotransmitters namely, glutamate, aspartate, GABA and glycine by HPLC-FLD). Each dialysate sample was mixed with homoserine solution (internal standard) and pre-column derivatized with o-phthaldialdehyde (OPA) before injection into a HPLC-FLD with an analytical column (Khongsombat et al. 2008).

Data Analysis

All data of neurotransmitters level were presented as means ± SEM. Student's unpaired *t-test* were used for comparison between two groups. For comparison among two groups, one-way ANOVA was used. Differences were considered statistically significant at $p < 0.05$.

Results

Alteration of cortical amino acid neurotransmitters under K^+- Evoked Cortical Spreading Depression

Increases of cortical amino acid neurotransmitters from their respective base line were clearly observed at 30, 60 and 90 minutes after the application of KCl in 0.5% CMC-treated rats. The most prominent increment was noted on the level of glutamate (Figure 1) in which the average level within the observation period of 90 minutes was 127.45±4.22% while corresponding values of aspartate, GABA and glycine

were found to be 110.15±4.22%, 124±4.22% and 113.66% ± SEM value %, respectively (Figure 2-4).

Effects of VPA on cortical amino acid neurotransmitter levels during K^+-Evoked Cortical Spreading Depression

In contrast to observation in 0.5% CMC-treated group, topical application of K^+ in rats pretreated with VPA (200 mg/kg) did not evoke any increment of any cortical amino acid neurotransmitters observed. Moreover, the levels of all amino acids in VPA-treated group were decreased below their respective base line. Average level of cortical glutamate within the observation period of 90 minutes was 68.44 ± 6.07%, whereas the corresponding values for aspartate, GABA and glycine were 83.43 ± 6.07%, 97.67 ± 6.07% and 87.70 ± 6.07%, respectively.

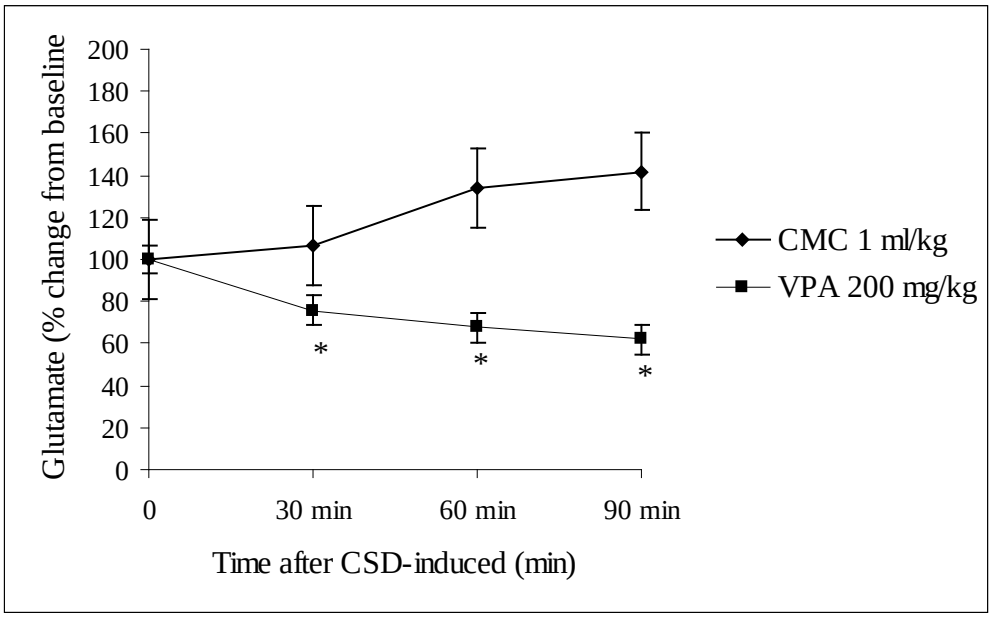

Figure 1. Effect of an intraperitoneal injection of CMC (n=6) and VPA (n=6) on level of extracellular glutamate (in percentage of basal level). Data is expressed in mean±S.E.M. The asterisks denote statistically significant difference from the corresponding value in CMC-administrated group. (p=0.05)

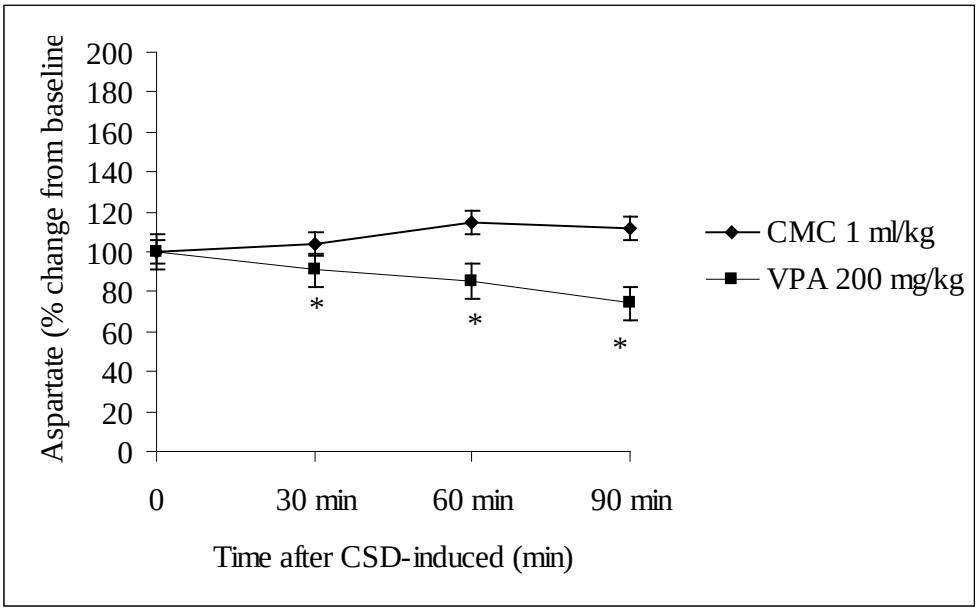

Figure 2 Effect of an intraperitoneal injection of CMC (n=6) and VPA (n=6) on level of extracellular aspartate (in percentage of basal level). Data is expressed in mean±S.E.M. The asterisks denote statistically significant difference from the corresponding value in CMC-administrated group. (p=0.05)

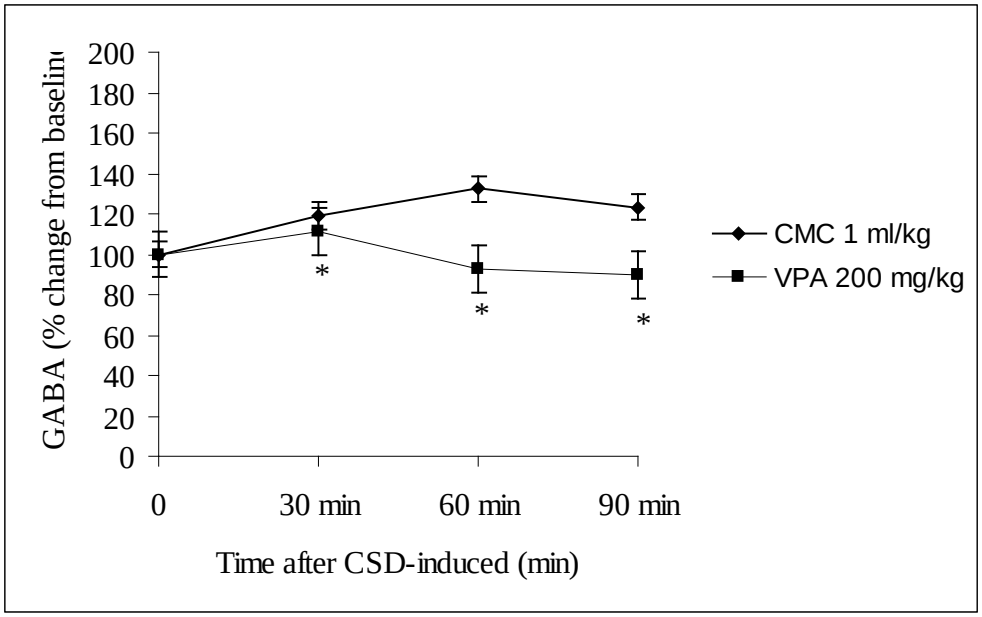

Figure 3 Effect of an intraperitoneal injection of CMC (n=6) and VPA (n=6) on level of extracellular GABA (in percentage of basal level). Data is expressed in mean±S.E.M. The asterisks denote statistically significant difference from the corresponding value in CMC-administrated group. (p=0.05)

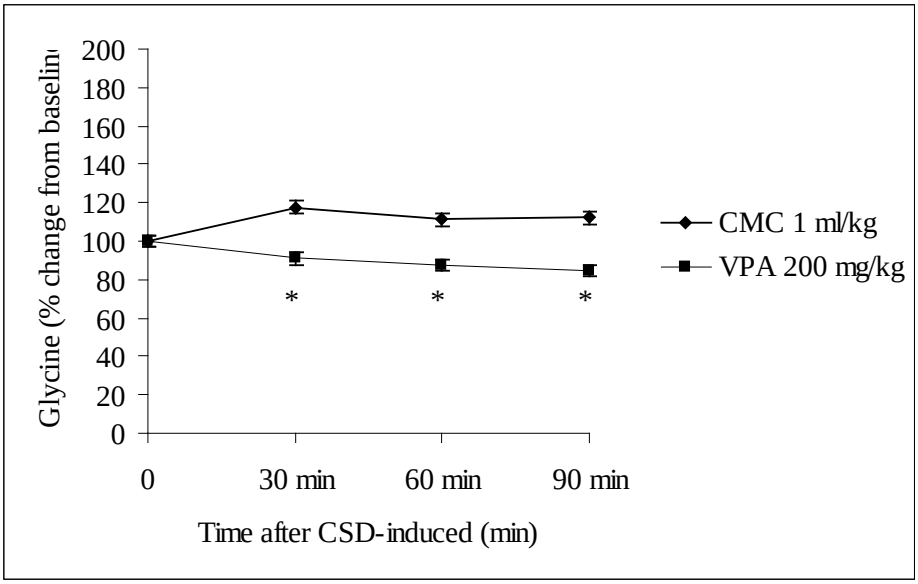

Figure 4 Effect of an intraperitoneal injection of CMC (n=6) and VPA (n=6) on level of extracellular glycine (in percentage of basal level). Data is expressed in mean±S.E.M. The asterisks denote statistically significant difference from the corresponding value in CMC-administrated group. (p=0.05)

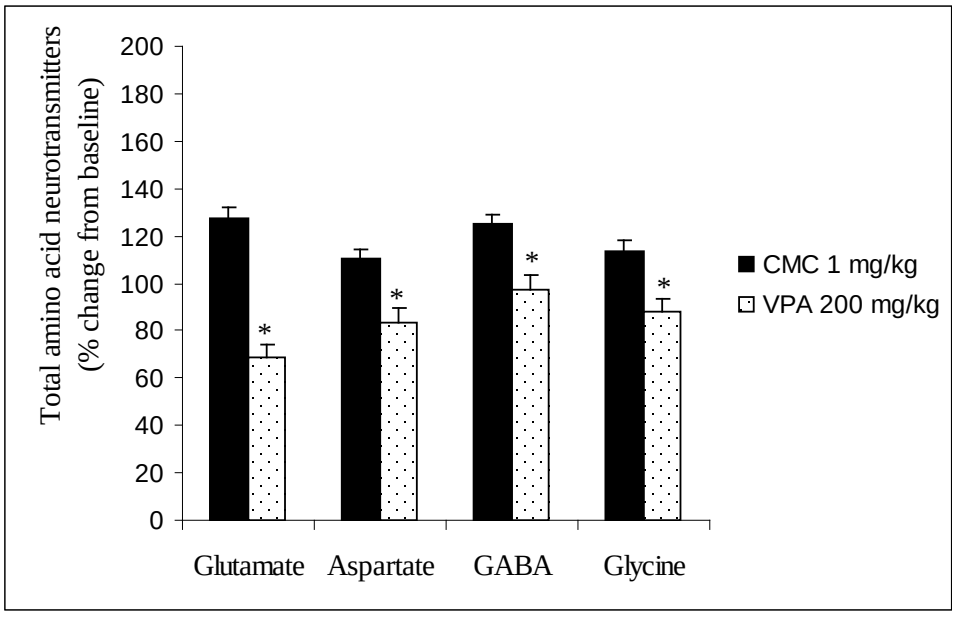

Figure 5 The effect of intraperitoneal injection of CMC and VPA on average total level of amino acid neurotransmitters from dialysate collected over 90 minutes after CSD induction. Data is expressed in mean ± S.E.M. Asterisks denote statistically significant difference between VPA and CMC (p=0.05)

Apparently, the levels of all amino acid neurotransmitters after the application of K^+ in VPA-treated group were significantly lower, at all time points than those found in 0.5% CMC-treated group under the same condition (Figure 5). However the greatest disparity was noted on the level of glutamate (127.45 ± 4.22% vs 68.44 ± 6.07 %) whereas smaller effect was noted on aspartate (110.15 ± 4.22% vs 83.43 ± 6.07%), GABA (124 ± 4.22% vs 97.67 ± 6.07%) and glycine (113.66 ± 4.22% vs 87.70 ± 6.07%).

Discussion

CSD is a phenomenon described by an expanding depolarization of neurons which can be found in many species. In human, CSD is suggested to underlie the aura or prodrome associated with an initiation of migraine (Kelman 2004). It is not clear how CSD could trigger migraine attack, but most factors facilitate CSD are excitatory events, such as NMDA receptor activation and seizures (Rice and Delorenzo 1998). CSD lead s to excessive release of various brain neurotransmitters including glutamate which recently has been proposed to play a key role in migraine. Therefore, glutamate antagonists were expected to become a future drug for the treatment of migraine (Peeters et al. 2007).

In the present study, increases of both cortical excitatory (glutamate, aspatate) and inhibitory (GABA and glycine) amino acid neurotransmitter levels were observed in CMC-treated rats after the application of K^+. Interestingly, the most pronounce effect was noted on glutamate which is a principal excitatory amino acid neurotransmitter in the brain. This could possibly explain the electrophysiological finding that induction of CSD increased frequency and amplitude of depolarization. VPA which has been previously found to suppress CSD spreading (Ayata et al. 2006) significantly decreased the level of all cortical amino acid neurotransmitters measured with greatest reduction on glutamate and lesser effect on the others. Similar profile of responses of cortical amino acid neurotransmitters to VPA have been demonstrated in rat receiving pilocarpine (Khongsombat et al. 2008) and spontaneously recurrent seizure rats (Khongsombat et al. 2010). Reduction of GABA was proposed by the authors to be accounted by activation of presynaptic $GABA_B$ receptor by the increased GABA resulting from inhibition of GABA amino-transaminase by VPA (Loscher et al. 1981, Gobbi and Janiri 2006). By analogy, the results of VPA on cerebral amino acid neurotransmitters observed in the present study are likely to be explained by the same manner. The paramount reduction of K^+-invoked released of glutamate in combination with a reduction of aspartate, albeit to a lesser extent, could counteract the reduction of inhibitory amino acid neurotransmitters and possibly underlie the previous observation that VPA decreased frequency and severity of CSD (Bogdanov et al. 2011). This finding might account for its efficacy in migraine prophylaxis.

Conclusion

This study is the first research to reveal the effect of VPA on brain amino acid neurotransmitters during CSD which may, at least, partly account for its efficacy in migraine prophylaxis.

Acknowledgenents

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