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Institut für Physiologie I
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Spreading depression triggers ictal activity in disinhibited hippocampal slices

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ZUSAMMENFASSUNG

Spreading depression triggert ictuale Aktivität im disinhibierten Hippocampus

Khalil Sheikh

Die enge Verwandtschaft zwischen der Spreading depression (SD) und experimenteller epileptischer Aktivität hat zu zahlreichen Untersuchungen zum Wechselspiel dieser zwei Phänomene geführt. Trotz dieser Untersuchungen in verschiedenen Tiermodellen, ist der genaue Zusammenhang zwischen SD und epileptiformer Feldpotentiale unklar. Daher wurde in der vorliegenden Arbeit die Interaktion von SD und experimenteller epileptischer Aktivität in hippocampalen Rattenhirnschnitten untersucht.

Nach 45-minütiger Superfusion der Hirnschnitte mit dem GABA A Rezeptorantagonist Bicucullin in sub-epileptogener Konzentration (2,5 micromol/l), wurde durch Induktion einer SD ictaforme Aktivität in allen untersuchten Schnitten hervorgerufen. Experimenteller epileptischer Aktivität, die durch SD hervorgerufen wurden, wurden durch den N-methyl-D-aspartat (NMDA)- Rezeptorantagonisten, DL-2-Amino-5-phosphonovaleronsäure (APV, 50 micromol/l); a-amino-3-hydroxy-5-methylisoxazole-4-propionsäure (AMPA)-Rezeptorantagoniost, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 micromol/l); levetiracetam (500 micromol/l); Kalziumkanalblocker, Nifedipin (40 micromol/l); und 4-Aminopyridin (4-AP, 50 micromol/l) beeinflusst. Diese Ergebnisse weisen darauf hin, dass die SD möglicherweise die neuronale Erregbarkeit in Hirnschnitten verstärkt und zu anfallsartiger Aktivität führt. Die Daten stützen die Hypothese nach der durch SD hervorgerufene ionische, metabolische und haemodynamische Veränderungen die Entstehung von Spitzenpotentialen bahnen, und daher möglicherweise eine Rolle in der Epileptogenese einnehmen.

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Abstract

The close kinship between spreading depression (SD) and experimental epileptic activity stimulated extensive investigation into the mutual relationship of these two phenomena. In spite of several investigations in different animal epilepsy models, the relationship of SD and epileptiform field potentials (EFP) is not clear. Therefore, the interaction of SD and EFP was investigated in rat hippocampal tissues. After superfusion of these slices with $GABA_A$ receptor antagonist bicuculline at sub-epileptogenic concentration (2.5 micromol/l) for 45 min, initiation of SD induced ictaform epileptic activity in all tested slices. EFP triggered by SD were affected by the application of N-methyl-D-aspartic acid (NMDA)-receptor antagonist, DL-2-Amino-5-phosphonovaleric acid (APV, 50 micomol/l)); a-amino-3-hydroxy-5-methylisoxazole-4- propionic acid (AMPA)-receptor antagonist, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 micromol/l); levetiracetam (500 micromol/l); calcium channel blocker, nifedipine (40 micromol/l); and 4-aminopyridine (4-AP, 50 micromol/l). The results indicate that SD may increase the neuronal excitability in neuronal tissue and trigger the seizure-like activities. These data support the hypothesis that the ionic, metabolic, and hemodynamic changes associated with the SD wave facilitate subsequent generation of high voltage spiking, and therefore may play a role in epileptogenesis.

Introduction

Spreading depression (SD) is a giant extracellular negative slow voltage which propagates in different brain structures at a very slow velocity (Leao 1947). A brief period of excitation heralds CSD which is immediately followed by prolonged nerve cell depression. SD's cellular correlate is a depolarization shift associated with asymmetric intra-/extracellular ion distribution and efflux of excitatory amino acids from nerve cells (Somjen 2001). Occurrence SD was demonstrated by in vivo and in vitro studies in human neocortical tissues (Fig. 1; Avoli et al. 1991, Gorji et al. 2001, Strong et al. 2002).

SD is an experimental reaction induced by local stimulation in most gray matter regions, e.g., in the cortex, the hippocampus and cerebellum of a variety of species. It appears first at the stimulated site and spreads out in all directions at the velocity of 2–3 mm/min, so that increasingly distant areas undergo successively a similar temporary depression. A necessary manifestation of SD is a propagating extracellular negative potential with an amplitude of 10–30 mV and a duration of more than 0.5–1 min, which may be preceded or succeeded by a positive deflection of variable amplitude and duration. Underlying this neuro-glial depolarization is a dramatic change in the distribution of ions between extra- and intracellular spaces. K^+ and H^+ release from the cells, while Na^+, Ca^{2+} and Cl^- enter together with water causing cells to swell and the volume of the extracellular compartment to be reduced. SD is accompanied by an increase of glucose utilization and O_2 consumption. Recovery of SD depends on energy metabolism (Gorji 2001).

This phenomenon has been studied in vivo in several animal species and in vitro in brain slices and in retinal preparations under various experimental conditions. It has been also observed in human neocortical tissue in vitro and in human hippocampus as well as striatum

and neocortex in vivo. However, there is an in vivo experiment which suggests that SD is more difficult to elicit in human than rodent cortex and may not occur in man (Somjen 2001).

SD can be regularly initiated if the tissue susceptibility is artificially raised. Hypoglycemia and hypoxia as well as changing the extracellular ionic micromilieu by applying solutions with increased K^+, decreased NaCl or with the Cl^- of the latter replaced by certain other anions lower the threshold. Conversely, the susceptibility of SD initiation is lowered or the occurrence of SD is prevented in previously susceptible tissue by solution with increased Mg^{2+} or NaCl, or with the Na^+ replaced by certain other cations. SD also is triggered by various modes of mechanical, chemical and electrical stimulation (Somjen 2001).

No explanation of the propagation of SD has been suggested that accounts for all the facts presently proven. The hypothesis that gained wide acceptance is that the spread of SD probably involves the release and diffusion of the chemical mediators, most likely K^+ and glutamate into the interstitial fluid. Given the widespread potential signalling capacities of Ca^{2+} waves, observations of the interactions between astrocytes and neurons in cell culture have suggested that Ca^{2+} waves play a role in SD initiation and propagation (Gorji 2001).

There is sufficient evidence to admit the SD plays an important role in different neurological disorders (Gorji, 2000). Subdural recordings in patients demonstrated that SD is critically involved in various disorders associated with acute neuronal injury including traumatic and spontaneous intracerebral haemorrhage (Strong et al., 2002; Fabricius et al., 2006) as well as subarachnoid haemorrhage and ischaemic stroke (Dreier et al., 2006) and contribute to tissue damage. Furthermore, propagation of a SD-like phenomenon in human neocortical tissues has been shown to generate aura symptoms in migrainous patients (Hadjikhani et al. 2001).

Although interrelation of SD and epilepsy has been considered for a long time, the possible pathophysiological role of SD in epilepsy needs to be elucidated.

Migraine and epilepsy are both disorders characterized by transient paroxysmal neurological dysfunction, usually with a normal neurological examination between attacks. The relationship between these disorders has long been suspected. The medieval Iranian practitioner, *Rhazes* (860–940 A.D.) defines a syndrome in which migraine headache and conjunctivitis preceding epileptic attacks in the headache section of his *Al-Hawi*. A number of syndromes in which migraine and epilepsy are related have been described. Headaches are observed quite frequently following epileptic attacks and seizures provoke a syndrome similar to the headache phase of migraine in 50% of epileptics. A number of anticonvulsive drugs have the capacity to stabilize migraine and some anti-migraine drugs increase the epilepsy threshold. Gabapentin is an antiepileptic drug of new generation that enhances brain GABA levels has an effective therapeutic action in the prophylactic treatment of migraine headache. First-generation antiepileptic drug, sodium valproate, is used for the treatment of pain for trigeminal neuralgia and migraine headache. Calcium channel blockers are effective drugs in prophylactic and acute treatment of migraine. Flunarizine produced significant seizure reduction in two trials in therapy-resistant patients. Aura disappeared and post-ictal headache ameliorated in refractive epileptic patients treated with flunarizine. Magnesium which ameliorates migraine headaches is an anticonvulsant compound in eclampsia. It was reported that combination therapy with anticonvulsant and anti-migraine drugs in some intractable epileptics improves seizure control.

rCBF changes in epilepsy have some similarities to those changes in migraine. The human brain frequently has been observed during convulsive seizure. An initial pallor preceding and during the early phase of epileptic attack was reported while the latter part of the fit and post-

convulsive state were accompanied by widespread vasodilatation of cerebral vessels. The dilated vessels were first cyanotic, and then for several hours bright red. Positron emission tomography shows a significant reduction of rCBF and oxygen consumption in interictal period and an increased local blood flow in the ictal state in epileptic focus. The small but significant reduction in both of those was observed in cerebral hemisphere homolateral to the hypoperfused and hypometabolic areas. Ictal scans revealed a focal or multifocal increase in rCBF and oxygen consumption in an active seizure focus.

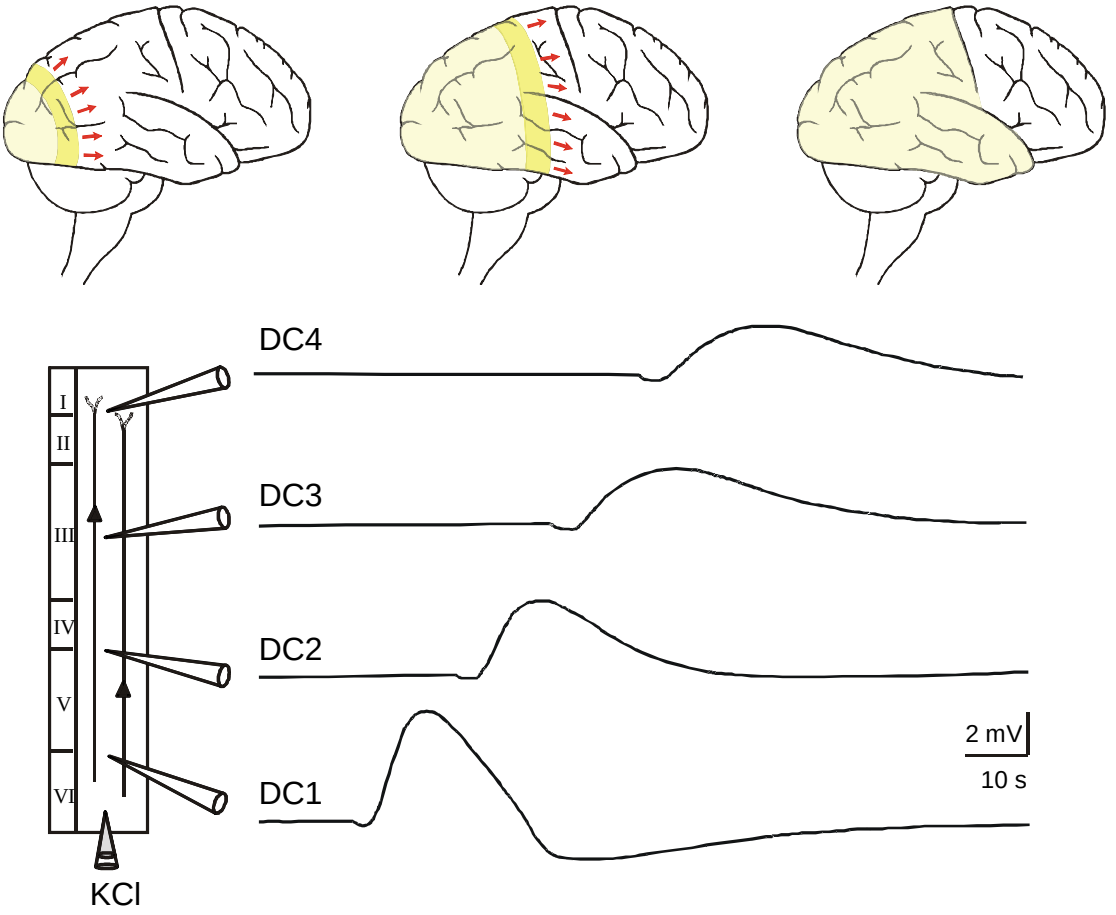

Fig. 1. Propagation of a negative DC-potential wave after injection of KCl in a neocortical slice. Injection of KCl solution (3 M) via a microelectrode elicited spreading depression-like fluctuation during superfusion with artificial cerebrospinal fluid. Injecting and recording electrodes arranged as shown. Voltage variations were recorded simultaneously by four electrodes (DC1–DC4) which set apart by 1 mm.

SD is a well-known phenomenon in experimental epilepsy. SD has been observed in a variety of in vitro and in vivo epilepsy models in different animal species. Reduction of extracellular Mg^{2+} concentrations, activation of NMDA receptors, blocking of K^+ channels, e.g., by 4-aminopyridine, increased extracellular K^+, blocking of Na^+–K^+ ATPase, e.g., by ouabain, blocking of Ca^{2+} channels, e.g., by $NiCl_2$, blocking of GABA receptors, e.g., by picrotoxin, are the common pathways for eliciting epileptiform burst discharges and SD in experimental models. By all aforementioned mechanisms SD appears spontaneously between epileptiform ictal events. SD can be elicited in susceptible area by a single discharge of an epileptic focus (spike triggered SD). Epileptiform field potentials usually suppress during SD occurrence and reappear in few minutes. CSD penetration into the epileptic foci was established in different model of epilepsy. However, it should be noted that SD does not enter electrically or pharmacologically elicited foci of epileptic activity with high rates of interictal discharges which resulted in anomalous SD propagation. This abnormal SD conduction may account for periodic changes of ictal and interictal activity found in some types of focal epilepsy.

Although in above-mentioned studies spontaneous SD is a concomitant phenomenon with epileptic burst discharges, inducing of SD by known stimulus such as KCl application drops the incidence of seizure in some in vitro and in vivo epilepsy models. CSD can reduce the seizure appearance in audiogenic epileptic seizure. Eliciting CSD prior to increasing of the susceptibility for audiogenic epilepsy, e.g., by metrazol decreases the seizure appearance and increases the latencies of the running fit onset. Functional decortications induced by KCl application abolish the epileptic bursts of generalized penicillin epilepsy in cats. Induction of unilateral SD completely suppresses the bilateral electrographic and clonic convulsive seizures and produces only a brief electrographic seizure by stimulation of the ipsilateral kindled amygdala. Interestingly, SD can also reduce the threshold for seizures under certain conditions and acts as an epileptogenic factor. SD may gradually change into spreading

convulsion when repeatedly evoked at brief intervals. Paroxysmal activity instead of depression appears in cortical regions topically treated with acetylcholine or pilocarpine when they are invaded by SD (Gorji 2001).

The first-generation anticonvulsant drug, phenytoin affects the SD elicited by mechanical or chemical stimulation in isolated chick retina. Phenytoin increases the threshold concentration of KCl to initiate this phenomenon; decreases the velocity of propagation and shortens the duration of the slow potential, ionic and volume changes of the extracellular space during SD.

Several characteristics of SD onset, propagation, and termination are similar to the activity that occurs during seizure episodes (for review see Gorji 2001, Somjen 2001). SD waves propagate across cortex with a speed similar to that of the Jacksonian march of clinical epilepsy (Leao 1972). There are several common pathways for induction of epileptiform burst discharges and of CSD in experimental studies. Co-occurrence of SD and epileptic activity has been observed in a variety of *in vitro* and *in vivo* epilepsy models in different animal species (Van Harreveld and Stamm, 1953; Koroleva and Bures, 1983) and in human neocortical tissue slices (Avoli et al., 1991; Gorji and Speckmann, 2004). SD can block or elicit epileptic burst discharges (Gorji et al. 2003, Van Harreveld and Stamm 1953) and can be triggered or blocked by epileptic activity in different animal models (Van Harreveld and Stamm 1955, Bures et al. 1975). Seizure activity may trigger CSD in rabbits (Van Harreveld and Stamm, 1955), and single cortical spikes may trigger CSD in rats (Koroleva and Bures, 1983). In rodents, CSD can both elicit and block epileptic discharges (Van Harreveld and Stamm, 1953; Gorji et al., 2003).

It has shown that SD enhances the repetition rate as well as the amplitude of spontaneous rhythmic discharges in neocortical tissues obtained from medically refractory epileptic patients. It was hypothesized that CSD may facilitate the synchronization of different foci of

rhythmic sharp field potentials and increases the excitability in human brain tissue (Gorji and Speckmann, 2004). One possibility that seizure activity may be enhanced by repeated CSD is selective suppression of GABAergic function (Kruger et al. 1996). Indeed, a recent investigation has shown that SD triggers ictaform discharges in disinhibited human neocortical slices obtained during epilepsy surgery (Gorji et al. 2004). In the present study, we have investigated the characteristic features of ictaform activities triggered by SD in disinhibited rat hippocampal tissues.

Material and methods

The experiments were carried out on medial hippocampal slices from adult rats (220–350 g). The brain was removed under deep methohexital anaesthesia. The hippocampi were dissected and cut into slices of 500 µm thickness. The slices were preincubated at 28°C for 60 min in artificial cerebrospinal fluid (ACSF). The ACSF contained (mmol/*l*): NaCl 124, KCl 4, $CaCl_2$ 1.0, NaH_2PO_4 1.24, $MgSO_4$ 1.3, $NaHCO_3$ 26 and glucose 10. The ACSF was continuously equilibrated by 5% CO_2 in O_2 and the pH stabilized at 7.35–7.4. After 30 min preincubation, $CaCl_2$ was elevated to 2.0 mmol/l. Slices were individually transferred to an interphase recording chamber, placed on a transparent membrane, illuminated from below and continuously perfused (1.5–2 ml/min) with carbogenated ACSF at 32 °C. A warmed, humified 95% O_2 and 5% CO_2 gas mixture was directed over the surface of the slices.

Extracellular field potentials were recorded with glass microelectrodes (150 mmol/L NaCl; 2–10 MO) connected to the amplifier by an Ag/AgCl–KCl bridge in the hippocampal CA1 and CA3 regions (stratum pyramidale). Field potentials were traced by an ink-writer and recorded by a digital oscilloscope.

Induction of SD

A glass electrode filled with 2 m KCl was fixed in a special holder connected with plastic tube to a pressure injector and the tip inserted into the hippocampal tissues (dentate gyrus). A high-pressure pulse was applied to inject in the tissue an amount of K^+ sufficient to induce SD (tip diameter, 2 µm; injection pressure, 0.5–1.0 bar applied for 200–300 ms, two separate injections, 1–3 nL per pulse, 2–5 mm apart from nearby recording electrodes). SD were evaluated with respect to their amplitudes, duration and velocity rates. Duration of DC potential fluctuation width was measured at its half-maximal amplitude.

Experimental protocols

Two experimental protocols were used, each of which consisted of several periods. The first experimental protocol consisted of four periods as follows: (a) control period, hippocampal slices were superfused with ACSF (30 min), tested for spontaneous SD or epileptiform field potentials (EFP); (b) KCl injection, induction of SD (SD1); (c) application of bicuculline (2.5 micromol/l, 45 min) before the second injection of KCl (SD2); (d) washout of bicuculline with ASCF (45 min, second control period), third injection of KCl (SD3).

The second experimental protocol consisted of four periods as follows: (a) control period, hippocampal slices were superfused with ACSF (30 min), tested for spontaneous SD or EFP; (b) application of bicuculline (2.5 micromol/l, 45 min) before the first injection of KCl (SD1); (c) addition of N-methyl-D-aspartic acid (NMDA)-receptor antagonist, DL-2-Amino-5-phosphonovaleric acid (APV, 50 micomol/l); a-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)-receptor antagonist, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 micromol/l); Dopamine D1 receptor agonist, SKF 81297 (50 micromol/l); levetiracetam (500 micromol/l); calcium channel blocker, nifedipine (40 micromol/l); or 4-aminopyridine (4-AP, 50 micromol/l) 45 min before the second injection of KCl (SD2); (d) washout of bicuculline with solution of period b (45 min), third injection of KCl (SD3).

Statistical analysis

All data are given as mean ± SEM. The data were statistically analysed using the Mann–Whitney Rank Sum test. Multiple comparisons were performed by analysis of variance test (ANOVA) for repeated measures followed by a Duncan's test. Significance was established when the probability values were less than 0.05. The investigations were approved by the local ethics committee (Ethikkommission der Ärztekammer Westfalen-Lippe und der Medizinischen Fakultät der Universität Münster; April 18, 2000, Reg.Nr.: OIVSpe).

Results

Triggering of epileptiform burst discharges by induction of SD

Application of KCl into hippocampal slices elicited deflections of the DC potentials consisting of initially negative shifts, followed by positive waves (Fig. 1A). The amplitude and duration of negative DC deflections were 12.6 ± 0.7 mV and 93 ± 12.4 seconds, respectively. The triggered wave propagated against the flow of the superfusate and reached first the nearby electrode, and a few seconds later the other electrode located closer to the inlet of superfusate in the chamber. The velocity of SD propagation was determined by dividing the distance between two microelectrodes by the interval of DC potential shift appearances. The velocity of vertical propagation of DC fluctuation was 3.3 ± 0.1 mm/min.

In all hippocampal slices (n = 25), induction of SD did not trigger any epileptiform field potentials (EFP; SD1, Fig. 1A). However, reduction of inhibitory tone of hippocampus by application of low concentration of $GABA_A$ receptor antagonist bicuculline leads to triggering of epileptiform field potentials (EFP) by induction of SD. After addition of bicuculline (1 micromol/l) to the superfusate for 45 minutes, induction of SD (SD2; amplitude: 11.8 ± 0.6 mV and duration: 101 ± 13.3 seconds) triggered EFP in all tested slices (SD2; n = 16). EFP appeared within 3.4 ± 0.6 minutes after initiation of negative DC fluctuation and vanished

after 23.4 ± 4.1 minutes. In five slices, however, EFP continued for at least 180 minutes before field potential recordings interrupted. The amplitude and duration of EFP were 0.96 ± 0.2 mV and 187 ± 32 msec, respectively. After washout of the bicuculline for 45 minutes, induction of SD (SD3; amplitude: 10.5 ± 0.8 mV and duration: 92 ± 10.3 seconds), in 90 % of the slices, was not followed by appearance of EFP (n = 21, SD3; Fig. 1B). In three slices, however, induction of SD after bicuculline washout in lesser extent evoked epileptiform discharges. The amplitude and duration of these potentials decreased to 74% and 53% of levels observed under bicuculline application, respectively. In control experiments, application of low dose bicuculline (1 micromol/l) for at least 150 minutes did not elicit any epileptiform potentials (Fig. 2; n = 10).

Pharmacological characteristics of SD triggering EFP

We studied the involvement of the different ionotropic glutamate receptors in the triggering of EFP by propagation of SD in the hippocampus. The roles of NMDA- and AMPA-subreceptors were tested by application of NMDA antagonist, APV (25 micromol/l, n = 8), or non-NMDA antagonist, CNQX (10 micromol/l, n = 9), respectively. Triggering of EFP by SD propagation was both NMDA- and non-NMDA-receptor dependent. Induction of SD under application of bicuculline triggered EFP in CA1 area. Administration of APV for 45 minutes before eliciting of the second SD in hippocampal tissues caused a marked reduction in the amplitude of SD and the duration of EFP. However, other characteristic features of EFP as well as SD were not affected by APV. Addition of CNQX to the perfusion solution for 45 min

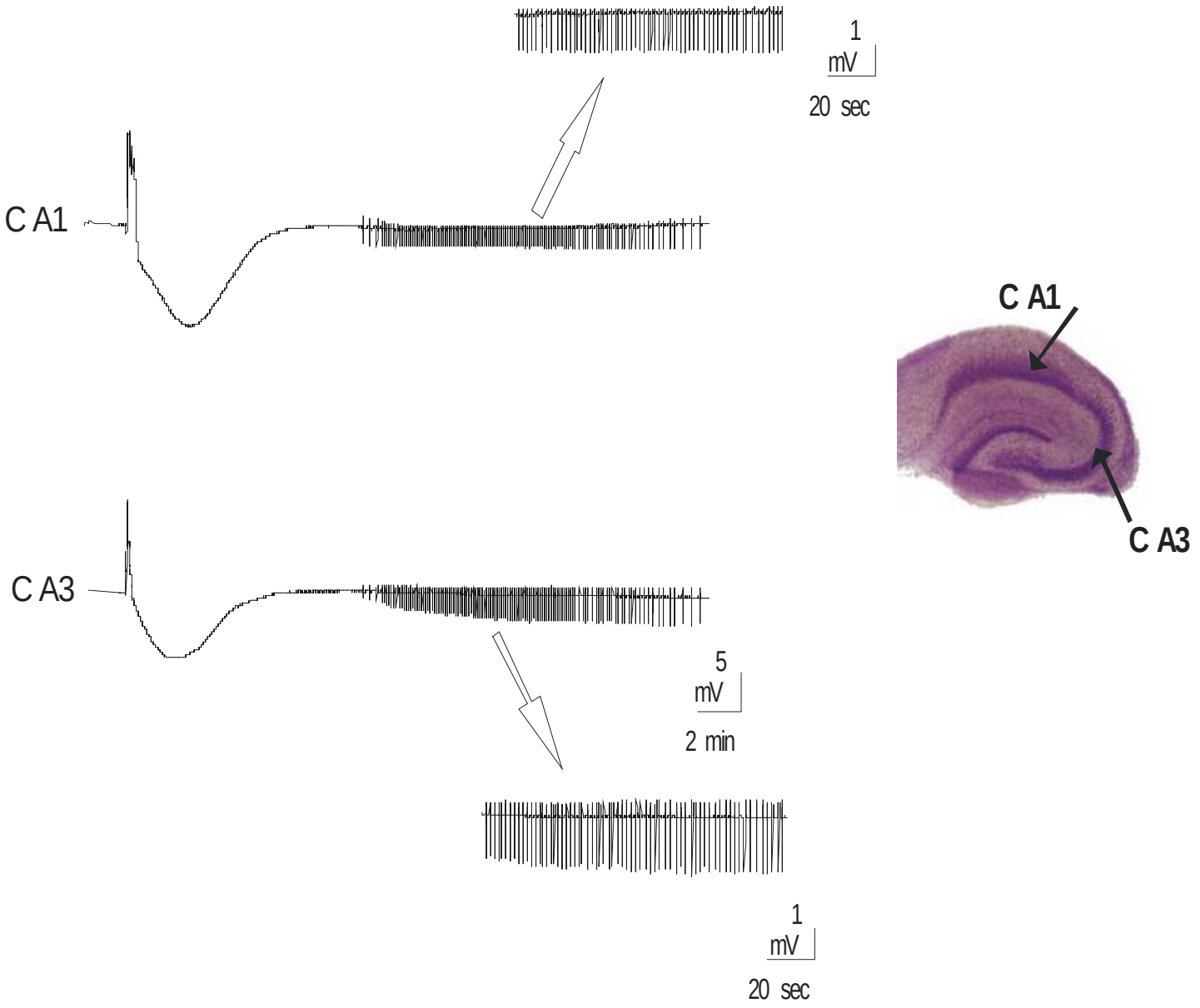

Fig. 2. Propagation of spreading depression (SD) followed by ictaform epileptic field potentials in rat hippocampal slices. Negative DC field potentials were recorded from CA1 and CA3 regions. Note the different time interval between SD and ictaform burst discharges appearance in CA1 and CA3 areas.

after initiation of the first SD resulted in reversible reduction of SD duration ($P = 0.001$, Fig. 4 A, B). In addition, CNQX also completely abolished appearance of EFP after initiation of SD. After 45 minutes of CNQX washout, induction of SD triggered EFP with the characteristic features similar to the first control period (Fig. 3).

Dopamine D1 receptor agonist SKF 81297 (50 micromol/l, n = 8)) was applied for 45 minutes after propagation of the first SD. Application of SKF 81297 did not affect EFP triggered by SD. However, SKF 81297 significantly increased the amplitude of the second SD (136 ± 5% control; $P = 0.001$). The amplitude of SD returned to control level after one hour washout of the substance. Furthermore, to study the effect of L-type calcium channel blocker on SD triggering EFP, nifidepine at concentration of 40 micromol/l was applied for 45 minutes before induction of the second SD. Nifedipine did not change the amplitude and duration of SD. In contrary, nifedipine significantly increased the repetition rate, the amplitude, and the duration of EFP. Nifedipine also significantly prolonged the duration of the appearance of EFP (fig. 3 and 4).

4-AP (10 micromol/l, n = 8) was added to the bath solution after the first SD triggered EFP for 45 minutes. Application of 4-AP has no effect on the amplitude and the duration of SD. 4-AP, however, reversibly increased the frequency rate as well as the amplitude of EFP but has no effect on the duration and prolongation of triggered EFP (Fig. 5). The effect of levetiracetam (500 micromol/l) was tested on SD triggering EFP in ten slices. In all slices, levetiracetam inhibited the triggering of EFP by induction of SD. The inhibitory effects of levetiracetam on EFP were completely reversible. Levetiracetam has no effect on SD's amplitude and duration (fig. 4). The summary of the effects of different substances on EFP triggered by SD was presented in Table 1.

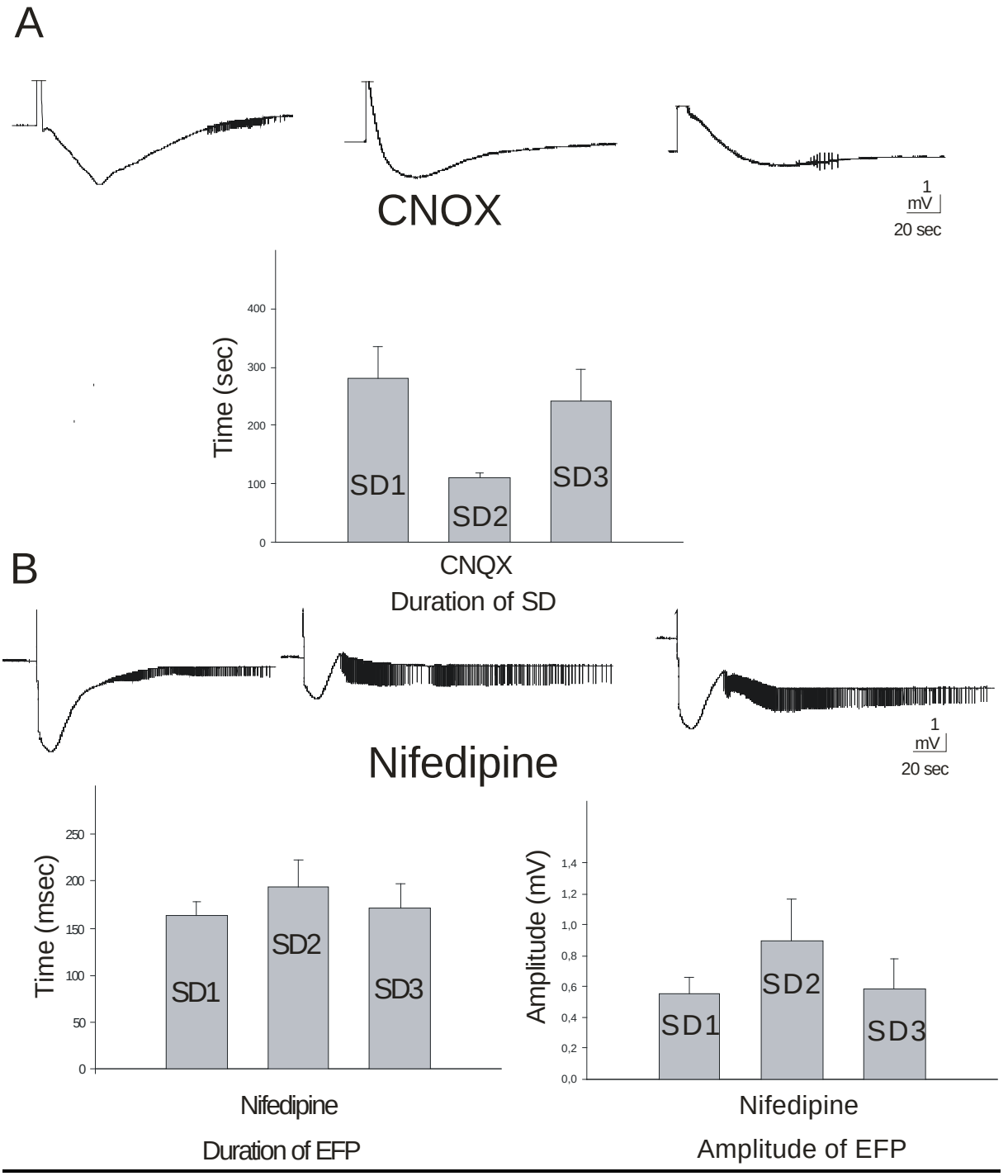

Fig. 3. The effect of blocking of AMP receptors by CNQX (A) and calcium channel blockers by nifedipine (B) on epileptiform field potentials triggered by induction of SD.

Fig. 4. The effect of antiepileptic drug levetiracetam, N-methyl-D-aspartic acid receptor antagonist, DL-2-Amino-5-phosphonovaleric acid (APV, 50 micomol/l), and Dopamine D1 receptor agonist, SKF 81297 (50 micromol/l) on epileptiform field potentials (EFP) triggered by induction of spreading depression (SD). The effect of levetiracetam on the latency of EFP appearance (A) and amplitude of EFP (B) as well as the effect of APV on latency of EFP appearance (C) and SKF on EFP amplitude were presented in bar diagram.

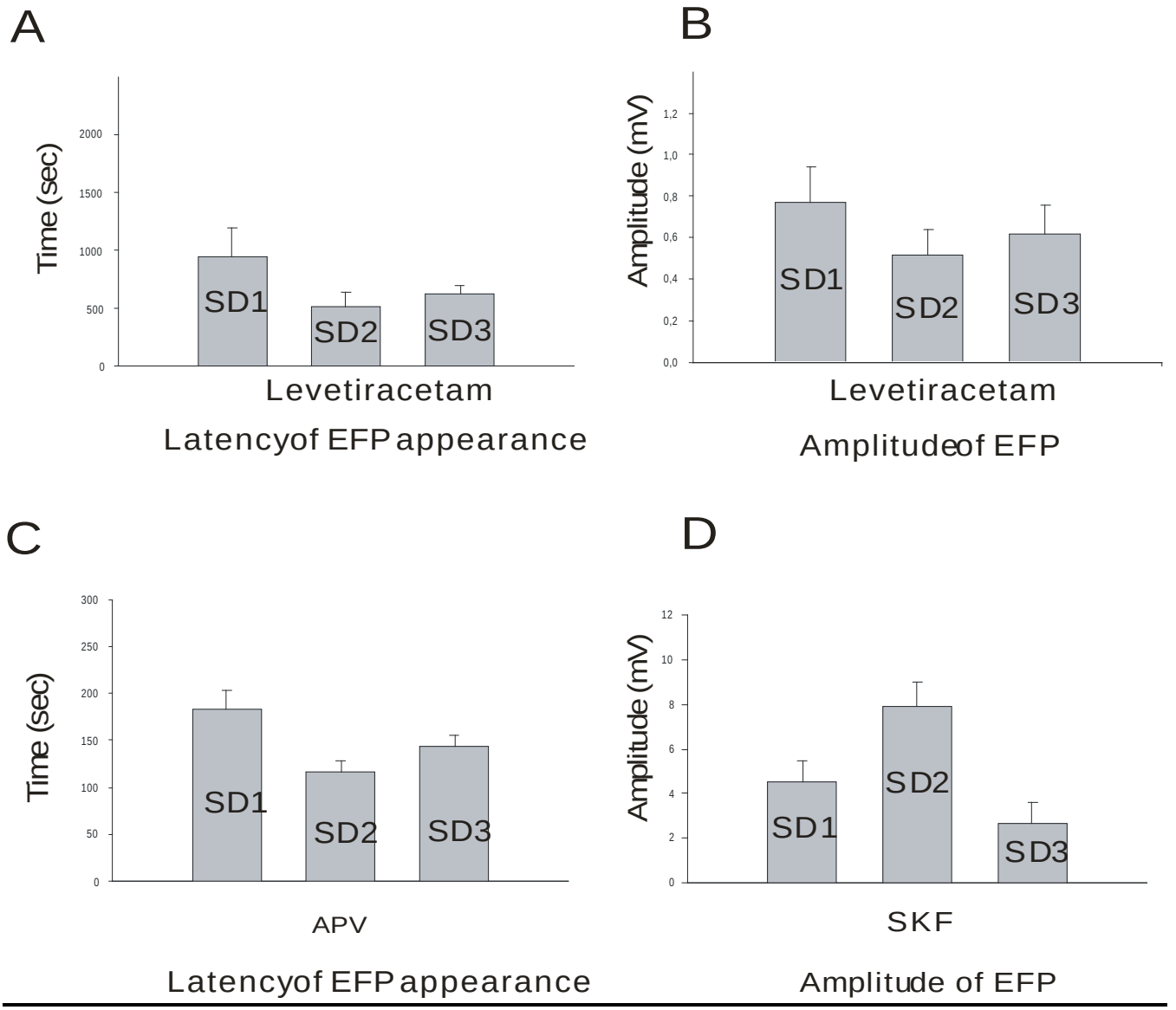

Fig. 5. The effect of 4-aminopyridine (4-AP) on ictaform activities triggered by spreading depression (SD. Note the significant enhancement of the repetition rate of epileptiform burst discharges by addition of 4-AP.

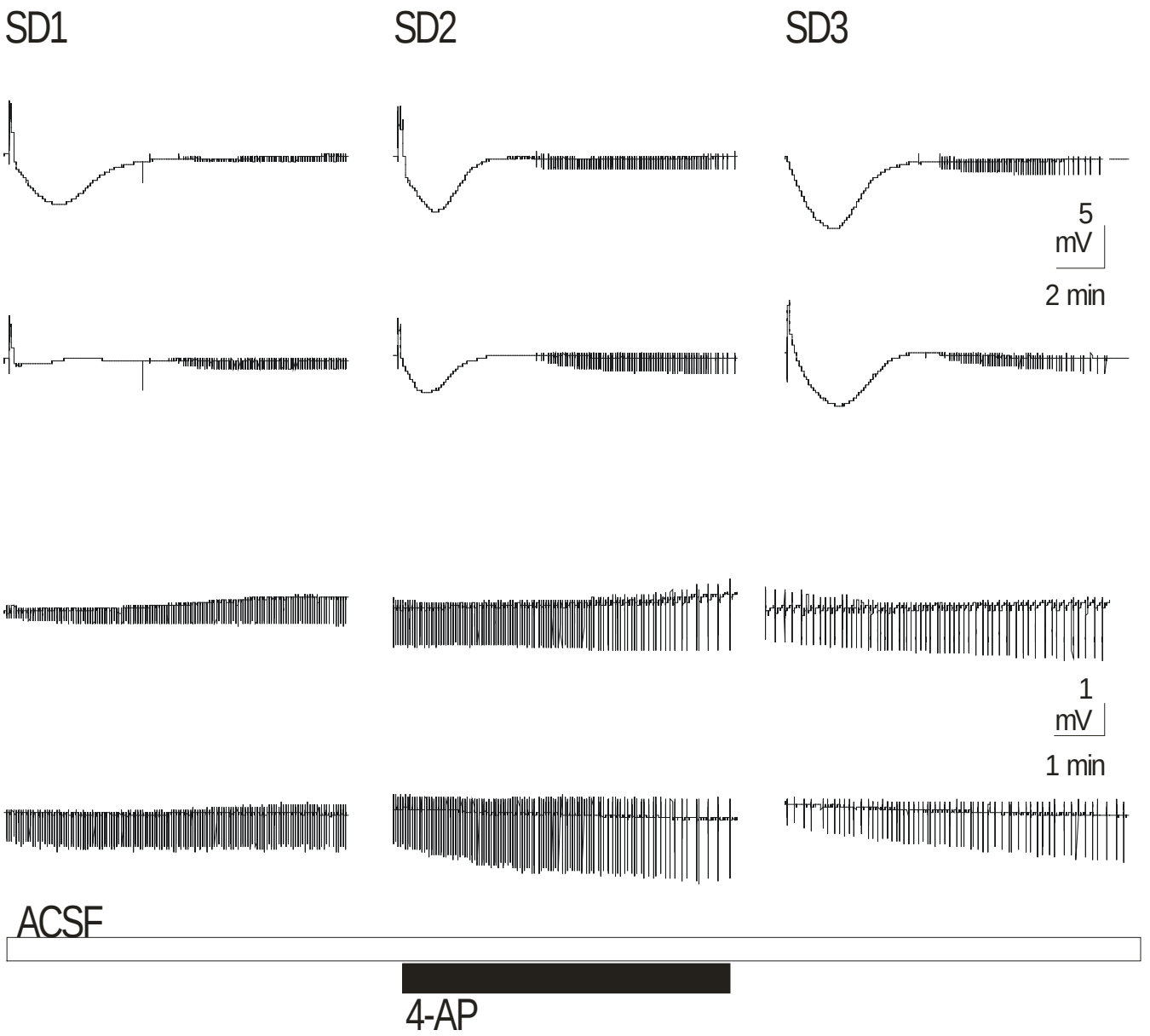

Table 1. The summary of pharmacological investigations of epileptiform field potentials (EFP) triggered by spreading depression (SD)

	SD Amplitude	*SD Duration*	*EFP Repetition Rate*	*EFP Amplitude*	*EFP Duration*	*Duration of Ictal Activity*
Levetiracetam	-	-	↓	↓	↓	↓
NMDA	↓	-	-	-	↓	-
AMPA	-	↓	↓	↓	↓	↓
Dopamin D1	↑	-	-	-	-	-
Ca^{2+} Channel	-	-	↑	↑	↑	↑
K^{+} Channel	-	-	↑	↑	-	-

Discussion

The present data indicate the potential of SD to triggering epileptiform burst discharges in disinhibited hippocampal tissues. Pharmacological study of SD triggering EFP revealed involvement of ionotropic glutamate receptors as well as L-type calcium channels and potassium current. Levetiracetam, a new antiepileptic drug, inhibits triggering of burst discharges by propagation of SD in the presence of subtle breakdown of inhibitory network.

SD cellular correlate is a depolarization shift associated with complete breakdown of the membrane potential that is dependent on the asymmetric intra-/extracellular ion distribution and is maintained by the energy consuming work of membrane pumps. The ability of neurons to generate action potentials is lost during SD, which explains the spreading electrical silence (depression of electrocorticographic activity) accompanied SD under physiological conditions (Somjen 2001). In the other hand, epileptic activity is characterized by the paroxysmal depolarization shift, a hypersynchronous network event resulting from a giant excitatory postsynaptic potential. A paroxysmal depolarization shift is the correlate of. The EPSP is presumably the consequence of synchronous activation of recurrent excitatory pathways (Jonston and Brown, 1984). Despite certain differences, SD facilitates the generation of epileptiform burst activities by enhancement of the neuronal synchronization in susceptible tissues (Gorji and Speckmann 2004). SD is accompanied by a very large increase in $[K^+]_o$. The increase in $[K^+]_o$ is accompanied by a precipitous drop in $[Cl^-]_o$, $[Na^+]_o$, and $[Ca^{2+}]_o$.

Paroxysmal depolarization shifts appear to spread between neurons similar to SD but at a significantly higher velocity (Goda et al., 2008). However, fast spreading components also exist in SD in form of inter-neuronal Ca^{2+} waves which precede the actual wave of DC

negative shift (Kunkler and Kraig, 1998). This fast interneuronal communication in the front of SD might have some relation to mechanisms of seizure spread.

In animal models, a transition of SD into convulsive pattern, moving at low velocity over the neocortex, has been produced by different methods in rabbits. Repetition of electrical stimulus eliciting SD caused spikes and high potential, which in further repetition developed into continuous convulsive pattern (Van Harreveld and Stamm 1953). Seizure-like SD in immature rabbit hippocampal slices was also reported (Haglund and Schwartzkroin 1984). Spreading convulsions in both in vitro experiments and subdural recordings appeared after SD in intractable epileptic patients (Gorji et al., unpublished data). In vitro studies revealed that reduction of inhibitory tone in neocortical neuronal network might play an important role in transition of SD into convulsive discharges. Impairment of GABA-mediated inhibition is believed to increase the susceptibility of neuronal tissues to both epileptogenesis and SD generation. Evidence from animal models, and from human studies, suggests that altered expression of GABA receptors may contribute to the pathophysiology of medically intractable epilepsy (Loup et al. 2000, Palma et al. 2002). Application of GABA blocks SD propagation (Ochs and Hunt 1960) and GABA receptor blockade elicits SD in neocortical tissues (Hablitz and Heinemann 1989). Some disequilibrium of the extracellular Cl^- distribution was observed in some patients with intractable epilepsy (Gorji et al, 2006). Perturbation of Cl^- homeostasis and GABAergic signalling in downstream regions are thought to facilitate induction of epileptic discharges (Hochmann et al. 1999, Cohen et al. 2003) and triggering of SD (Lenz et al. 1997, Muller 2000). Furthermore, it was shown that SD is accompanied by a transient rise in extracellular potassium concentrations to a level of 50 mM, which declined at a comparatively slow rate and returned to its pre-SD level in few seconds (Gorji et al. 2001). Increasing of potassium concentration may further impairs GABA mediated inhibition and leads to appearance of ictaform burst discharges. Higashima et al. (1996) have shown that

activation of GABAergic mechanisms is necessary for the generation of afterdischarges recorded in hippocampal slices after electrical stimuli. Experimental and computational data obtained by Traub et al. (1996) also suggest a role played by $GABA_A$-mediated depolarizing conductance in the epileptiform synchronization that occurs in some models of epileptiform discharge (in particular that induced by 4AP application). GABAergic inhibitory networks can also synchronize principal cells in the neocortex and hippocampus (Cobb et al. 1995). Higashima et al. (1996) have shown that activation of GABAergic mechanisms is necessary for the generation of afterdischarges recorded in hippocampal slices after electrical stimuli. Experimental and computational data obtained by Traub et al. (1996) also suggest a role played by $GABA_A$-mediated depolarizing conductance in the epileptiform synchronization that occurs in some models of epileptiform discharge (in particular that induced by 4AP application). GABAergic inhibitory networks can also synchronize principal cells in the neocortex and hippocampus (Cobb et al. 1995).

Activation of cholinergic systems and systemic enhancement of carbon dioxide causes the acute transition of SD into a spreading convulsive pattern in rabbit neocortical tissues (Van Harreveld and Stamm 1953). Simultaneous appearance as well as slowly propagated SD-like waves in patients suffering from cerebrovascular disease was also reported (Strong et al. 2002).

Human diseases for which CSD has relevance

There is now increasing evidence from subdural recordings in patients that CSD is critically involved in various disorders associated with acute neuronal injury including traumatic and spontaneous intracerebral haemorrhage (Strong et al., 2002; Fabricius et al., 2006) as well as subarachnoid haemorrhage and ischaemic stroke (Dreier et al., 2006). Furthermore, it is

assumed that CSD is the pathophysiological correlate of the migraine aura (Hadjikhani et al., 2001). Observations also indicate that SD is a clinical phenomenon. Sramka et al. (1977) obtained the first direct evidence for a SD in human grey matter in vivo. Mayevsky (1996) observed repetitive episodes of SD in a head injured patient.

Propagation of SD-like waves in human neocortical tissues generates aura symptoms in patients with migraine (Hadjikhani et al., 2001). Recently, Strong et al. reported the occurrence of transient episodes of depressed ECoG activity that propagated across the cortex at rates which is characteristic of SD. This group also observed transient depressions of ECoG amplitude that appeared essentially simultaneous in all recording channels, without clear evidence of spread after major traumatic or ischemic brain injury in humans. In line with these investigations, some *in vitro* studies demonstrated that SD occurred in human neocortical tissues (Gorji et al., 2001). DC fluctuations characteristic for SD occurred by exposing brain tissues excised for treatment of refractory epilepsy to ACSF with a reduced concentration of Mg^{2+} (Avoli et al. 1991), or low concentrations of amiloride or Ni^{2+} (Gorji et al. 20001). A recent in vivo study on human brain slices revealed the enhancement of spontaneous sharp field potentials frequency and amplitude due to SD propagation (Gorji and Speckmann 2004). It was suggested that occurrence of SD in cerebral tissue of epileptic patients, may enhance synchronization of neurons located in different foci of synchronous rhythmic sharp discharges and leads to a higher excitability. Therefore, from the data here, it may be concluded that SD enhance the excitability in the area it is propagated and lower the threshold for appearance of epileptiform burst discharges in susceptible zones.

Co-occurrence of CSD and epileptic seizures inexperimental models and humans

In the evolution of the central nervous system, the high degree of organizations in a state of unstable balance has produced the side effect of two principal pathological, hypersynchronous

network events: cortical spreading depolarization (CSD) and epileptic activity. The hallmark of CSD is a giant extracellular negative slow voltage variation of about 20mV which propagates in the cortex at a rate of 2-3mm/min. (Somjen, 2001). Its cellular correlate is a depolarization shift associated with complete breakdown of the membrane potential that, under normal conditions, is dependent on the physiological, asymmetric intra-/extracellular ion distribution and is maintained by the energy consuming work of membrane pumps such as the Na, K-ATPase. Maintenance of the membrane potential is a necessary premise for the ability of neurons to generate action potentials. Accordingly, this ability of neurons is lost during CSD which explains why spreading electrical silence accompanies CSD (Leao, 1947).

In contrast, epileptic activity, on the cellular level, is characterized by the so called paroxysmal depolarization shift. A paroxysmal depolarization shift lasts for 80 to 250ms and is the correlate of a hypersynchronous network event resulting from a giant excitatory postsynaptic potential (EPSP). The giant EPSP is presumably the consequence of synchronous activation of recurrent excitatory paths (Jonston and Brown, 1984). A high frequency series of action potentials rides on the paroxysmal depolarization shift which is later followed by hyperpolarization (Witte et al. 1989). Paroxysmal depolarization shifts appear to spread between neurons similar to CSD but at a significantly higher rate (Buchheim et al. 2002; Weissinger et al., 2005). Therefore, the mechanisms of spread are likely different. On the other hand, fast spreading components also exist in CSD in form of interneuronal Ca^{2+} waves which precede the actual wave of CSD (Kunkler and Kraig, 1998). This fast interneuronal communication in the front of CSD might have some relation to mechanisms of seizure spread.

The electrocorticographic (ECoG) correlate of CSD is a large slow potential change propagating at a rate of ~3mm/min. This is associated with spreading depression of any wave, burst or spiking alternating current activity with the consequence of transient or persistent

silence of ECoG activity. This pattern has now been demonstrated clearly in the human brain *in vivo* using subdural electrode strips in patients with spontaneous and traumatic intracerebral hematoma (Strong et al., 2002; Fabricius et al., 2006) as well as subarachnoid hemorrhage and ischemic stroke (Dreier et al., 2006). Furthermore, indirect evidence from studies with single photon emission computed tomography; positron emission computed tomography and functional magnetic resonance imaging strongly supported the hypothesis of Leao and Morison (1945) that CSD is the pathophysiological correlate of the migraine aura (Lauritzen, 1994; Hadjikhani et al., 2001).

In contrast, the ECoG correlate of paroxysmal depolarization shifts are epileptic interictal spikes. Epileptic seizure activity results from longer cellular depolarisations which are probably the consequence of a melting of paroxysmal depolarisations shifts. Usually, interictal and ictal epileptic ECoG activity is associated with higher amplitudes than normal activity which represents a practically useful difference from CSD.

However, a few early *in vivo* studies in neocortex of rabbits (Leao, 1947; van Harreveld and Stamm, 1953) and some more recent studies in acute juvenile rat hippocampal slices (Haglund and Schwartzkroin, 1984; Haglund and Schwartzkroin, 1990; Psarropoulou and Avoli, 1993) as well as juvenile rat hippocampal slice cultures (Kunkler and Kraig, 1998) reported a phenomenon with both features of CSD and epileptiform activity. This phenomenon was characterized by superimposed epileptiform discharges during the falling phase of the slow negative voltage variation of CSD and most notably during the recovery phase back to baseline. Pomper et al. (2005) found that a cluster of these CSD-like epileptiform events was associated with progressive cellular damage despite normoxic conditions. However, a possible importance of the above results to any clinical scenario remained questionable since this subtype of CSD has never been found in human tissue until now. Here we not only demonstrate for the first time such CSD-like epileptiform events in a

significant proportion of neocortex slices from patients with pharmaco-resistant focal epilepsy but also show its spontaneous and repetitive occurrence in a patient with acute subarachnoid hemorrhage *in vivo* who developed a grand mal seizure several weeks after the hemorrhage.

Conclusion

The data present direct electrophysiological evidence for triggering of epileptiform burst discharges by the propagation of SD in disinhibited hippocampal slices. Similar pattern of bioelectrical activity was observed in *in vitro* experiments upon human neocortical slices. The present data determined to what extent the large body of knowledge about SD and epilepsy in animal models can applicable to acute pathology in the epileptic brain. The clinical impact of the interaction between SD and ictal activity will be the subject of future investigations.

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