Aus dem Universitätsklinikum Münster

Institut für Physiologie I
Direktor: Univ.-Prof. Dr H. C. Pape

The effects of dopamine receptors on spreading depression in rat neocortical tissues

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ZUSAMMENFASSUNG

Einfluß von Dopamin auf die Spreading depression

Anna Maria Haarmann

Bei der spreading Depression(SD) handelt es sich um eine, sich mit einer Geschwindigkeit von 2-5mm/ min über den gesamten Cortex ausbreitende Depolarisationswelle, die von einer Abflachung bioelektrischer Aktivität begleitet wird.

Es ist anzunehmen, dass die spreadind depression Einfluss auf mehrere klinische Symptome wie Migräne, Kopfschmerzen oder vorübergehende globale Amnesie hat.

Dopamin ist ein essentieller Neurotransmitter, der wichtige modulierende Aufgaben übernimmt. Bei Patienten, die an Migräne leiden, besteht eine Hypersensivität für Dopamin, viele dafür typische Symptome können durch diesen Neurotransmitter getriggert werden.

Spreading depression führt zu einer Freisetzung von Dopamin .

Offensichtlich besteht also ein Zusammenhang zwischen spreading depression mit der konsekutiven Freisetzung von Dopamin und den klinischen Manifestationen der Migräne.

Die vorliegende Arbeit untersucht, in wie weit Dopaminagonisten bzw. – antagonisten Einfluß auf die spreading depression nehmen.

Die Experimente wurden am somatosensorischen Neocortex erwachsener Ratten durchgeführt.

Dabei wurden extrazelluläre Feldpotentiale abgeleitet und durch die Applikation von KCL eine spreading depression ausgelöst. Anschließend wurde der Einfluß sowohl von D2 Antagonisten als auch –Agonisten auf die SD untersucht.

Eine anschließende Versuchsreihe untersuchte den Einfluss von D2 Antagonisten bzw.-Agonisten auf evozierte exitatorische postsynaptische Feldoptentiale (EPSP), um so zu klären, ob ein Einfluss auf die längerfristige Modifikation synaptischer Übertragung besteht.

Die Resultate aus oben beschriebenen Versuchen ergaben, dass die Inhibition von D2 Rezeptoren- im vorliegendem Fall durch Sulpiride- zu einer signifikanten Suppression der spreading depression führt. Auch die synaptische Übertragung wird durch D2 Antagonisten beeinflusst.

Zusammenfassend ist festzuhalten, dass die Blockade von D2 Rezeptoren eine wichtige Rolle in der Behandlung von z. B. Migräne mit Aura spielt, der therapeutische Effekt mag in der Inhibition der neocortikalen SD liegen.

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Introduction

Spreading depression (SD) is a propagating wave of depolarisation associated by a depression of the neuronal bioelectrical activity for a period of minutes. SD could be initiated by different methods in animal models. SD is an "all-or-none" type process and propagates in the manner of a wave through gray matter. SD 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 crucial manifestation of SD is a propagating 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 fluctuation of variable amplitude and duration. Underlying this cellular depolarisation is a dramatic change in the distribution of micromilieu ions between extra- and intracellular compartments. Potassium and proton release from the cells, while sodium, calcium and chloride enter together with water causing cells to swell and the volume of the extracellular compartment to be decreased. SD is accompanied by an increase of glucose utilization and O_2 consumption. Recovery of SD depends on energy metabolism.

The first paper on SD, titled "Spreading depression of activity in the cerebral cortex" appeared in 1944, written by a young Brazilian investigator, Aristides Leão, working at the Harvard laboratory. Leão wanted to study the electrocorticogram (ECoG) of experimental epilepsy in anesthetized rabbits, but he was distracted from his original goal by an unexpected flattening of the ongoing normal bioelectrical activity that took the place of the anticipated epileptiform field potentials.The silencing of the ECoG trace crept slowly over the cortex, from one recording electrode pair resting on the cortical surface to the one beside it. According to Leão, SD and propagating focal seizures were related phenomena, generated by the same cellular elements, an inference later supported by others.

This phenomenon has been studied in vivo in several animal species and in vitro in brain slices and in retinal preparations under different 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. 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.

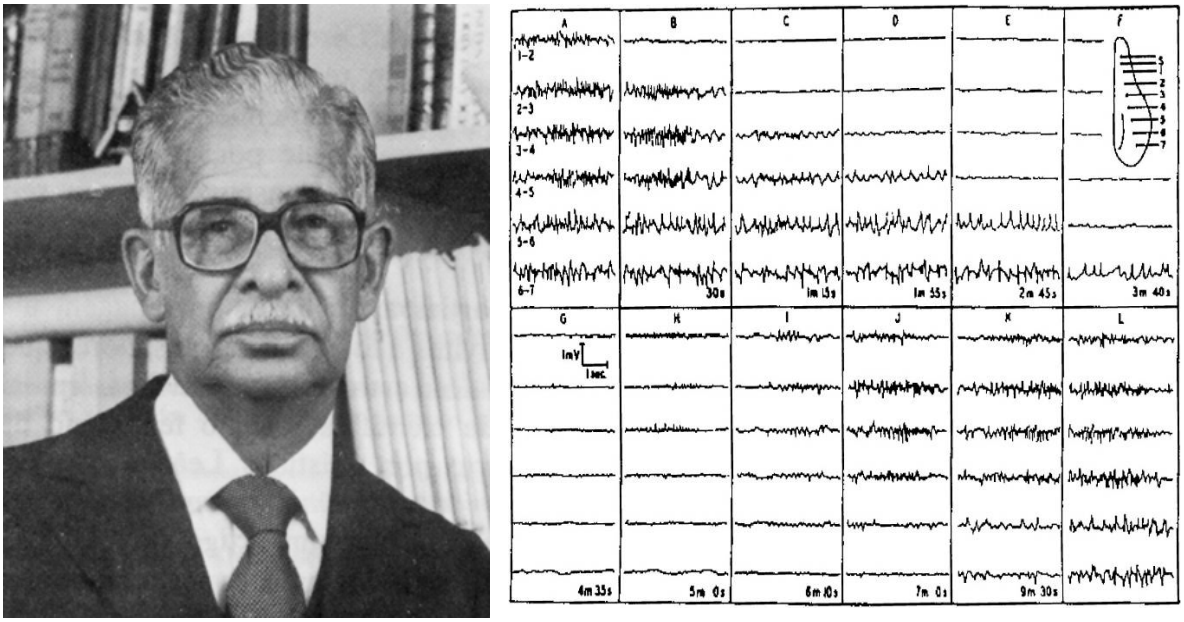

Fig. 1: Aristides Azevedo Pacheco Leão. Journal of Nourophysiology, 1944, Changes of bioelectrical activities recorded from a rabbit during propagation of spreading depression (SD). Traces reveal propagating flattening of epileptiform field potentials induced by spreading of cortical SD as well as propagating recovery of these activities.

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.

The unparalleled increase in extracellular potassium concentration ($[K^+]_o$) is accompanied by a precipitous drop in extracellular chloride concentration ($[Cl^-]_o$), extracellular sodium concentration ($[Na^+]_o$), and extracellular calcium concentration ($[Ca^{2+}]_o$), suggesting that K^+ leaving neurons is exchanged against Na^+ and Ca^{2+} that are entering and increased up to 60 mM. $[Ca^{2+}]_o$ decreases from its normal level of 1.2-1.5 mM to <0.3 mM. Cations are not exchanged one for one between intra- and extracellular solutions, for the reduction in $[Na^+]_o$ is greater than the increase in $[K^+]_o$. The concomitant drop in $[Cl^-]_o$ indicates that some of the Na^+ entering the cells is accompanied by Cl^-. It has been suggested that the deficit in extracellular anions is made up by anions leaving the cytosol. Organic anions, including glutamate, have been shown to be released during SD, although some of the glutamate originates from glial cells. An exact and complete balance sheet of all ingredients displaced during SD is yet to be completed, however, so much larger than that of the interstitial

compartment that neurons need to give up but a fraction of the K^+ they contain to achieve a many fold rise in $[K^+]_o$. Calculations based on the simultaneously recorded levels of $[Na^+]_o$ and $[K^+]_o$ and the known fractional volume of the interstitial space in hippocampal tissue indicate that a much reduced but still substantial trans-membrane potassium concentration gradient remains standing during SD.

The unusual magnitude of the changes in extracellular ion concentrations created the impression that intra- and extracellular ion concentrations equilibrate during SD, and this idea was bolstered by the nearly complete depolarization of neurons during SD. The volume of the cytosol is, however, so much larger than that of the interstitial space that cells need to give up but a fraction of the K^+ they contain to achieve a many fold rise in $[K^+]_o$. Calculations based on the simultaneously recorded levels of $[Na^+]_o$ and $[K^+]_o$ and the known fractional volume of the interstitial space in hippocampus indicate that a much reduced but still substantial transmembrane K^+ concentration gradient remains standing during SD.

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 propagation of SD probably involves the release of different 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 may also play a role in SD propagation.

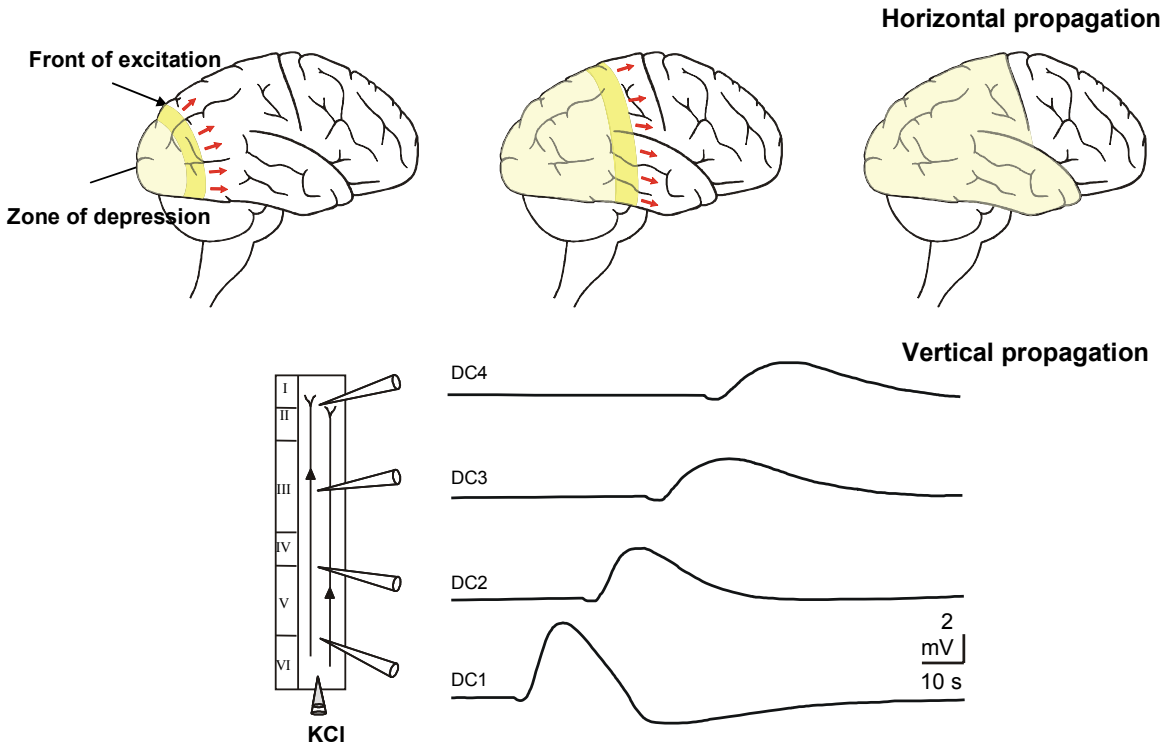

Figure 2. Propagation of cortical spreading depression and its electrophysiological recordings.

Clinical relevance of SD

SD belongs in the domain of the pathophysiology of the brain, and there are reasons to believe that it is involved in different clinical disorders, including migraine, cerebrovascular diseases, head injury and transient global amnesia. Processes similar to spreading depression in animal cortex are thought to take place in a number of pathological conditions in humans. These disorders include brain trauma, ischemia/infarction, migraine, epilepsy, hemorrhage and transient global amnesia. Direct alterations of electrical activity of cortical neurons by the locally spreading wave can lead to clinical symptoms (e.g. the aura phase of migraine). These same neuronal processes can also alter the neurochemistry of subcortical structures, modulating oxygen distribution, cell survival in these structures and behavior. This problem

has not been addressed before, mainly because SD induces such a short depression in each cortical loci, that transient neurochemical changes cannot be examined with conventional approaches, for example by microdialysis.

The designation migraine with aura denotes the syndrome of headache associated with characteristic sensory, motor, or visual symptoms, usually gradually developed over 5–20 min and lasting less than 60 min. The most common symptoms in aura phase are visual arising from dysfunction of occipital lobe neurons. The positive (stimulative) neurological symptoms, e.g., flashing lights are usually followed by negative (suppressive) ones, e.g., scotoma or hemianopia in this phase. Magnetoencephalographic studies in human revealed that the magnetic signals were seen in migraine patients but not in patients suffering from other forms of headache or normal controls. Three distinctive signal patterns; suppression of spontaneous cortical activity, slow field changes and large-amplitude waves, were observed strictly in migraine patients. In some migraine patients, magnetic signals were also recorded between attacks. The same magnetic fields appeared during the propagation of SD in the cortex of anesthetized animals. High-field functional MRI was used to detect blood oxygenation level-dependent (BOLD) changes during visual aura in three migraineurs. A focal increase in BOLD signals developed first in extrastriate cortex and spread at the velocity of 3.5 ± 1.1 mm/min over occipital cortex. These initial BOLD features were consistent with scintillations and paralleled by decreases in the stimulus-driven MR oscillations. Increasing in BOLD signals was followed by a decrease in the mean signal. This phase appeared to correspond to the localized scotoma and MR stimulus-induced response remained suppressed. Within 15 ± 3 min, both BOLD signals and MR stimulus-induced response recovered. During periods with no visual stimulation, but while the subject was experiencing scintillations, BOLD signal followed the retinotopic progression of the visual percept. Spreading BOLD signal changes as neocortical SD did not cross prominent sulci.

Recent investigations provide early insights into mechanisms that lead to trigeminovascular activation. SD is the first endogenous event identified upstream to trigeminovascular activation that appears to be noxious in experimental models. Neocortical SD, originally described by Leão. The slow spread of SD at 3–5 mm/min matches the propagation velocity of wave fronts in the Belousov–Zhabotinsky reaction, that is, a thermodynamic chemical reaction that shows the properties of a nonlinear chemical oscillator even in a Petri dish.

Unlike an epileptic seizure, which spreads asynchronously to activate adjacent brain, SD begins within a synchronously activated brain space.

In experimental animals, SD stimulates ipsilateral trigeminal axons that surround cortical blood vessels. SD causes a breakdown of the blood–brain barrier by mechanisms dependent on matrix metalloproteinase-9. Furthermore, neocortical SD causes ipsilateral extravasation of plasma proteins in dura mater, serving as an experimental marker of trigeminal nerve activation; it also induces c-Fos expression within the trigeminal nucleus caudalis. These findings and a transcription MRI study suggest that intense cortical perturbations like repeated SD can open the blood–brain barrier, thereby activating the trigeminovascular system. SD releases chemicals such as H^+, K^+, nitric oxide, and neurotransmitters into the extracellular space. It has been hypothesized that released molecules reach the pial surface by diffusion and accumulate in proximity to trigeminovascular afferents. Extracellular K^+ levels about 60 mmol/l were measured in the pial space during SD.

Consistent with an upstream role for SD, prolonged application of migraine prophylactic drugs suppresses SD in rats as a proposed mechanism of action. In line with the growing clinical recognition that prolonged administration of prophylactic drugs is important to achieve maximum therapeutic efficacy, treatment extension beyond 3–4 weeks also maximizes the inhibitory effects of topiramate, valproate, methysergide, amitriptyline, and propranolol on SD.

Dopamine

Dopamine is an essential neurotransmitter in a wide variety of animals, including both invertebrates and vertebrates. In the brain, this phenethylamine functions as a neurotransmitter, activating the five types of dopamine receptors; D_1, D_2, D_3, D_4 and D_5, and their variants. Dopamine is produced in several areas of the brain, including the substantia nigra and the ventral tegmental area Dopamine is also a neurohormone released by the hypothalamus.

Dopamine has many functions in the brain, including important roles in behavior and cognition, motor activity, motivation and reward, inhibition of prolactin production (involved in lactation), sleep, mood, attention, and learning. Dopaminergic neurons (i.e., neurons whose primary neurotransmitter is dopamine) are present chiefly in the ventral tegmental area of the midbrain, the substantia nigra pars compacta, and the arcuate nucleus of the hypothalamus

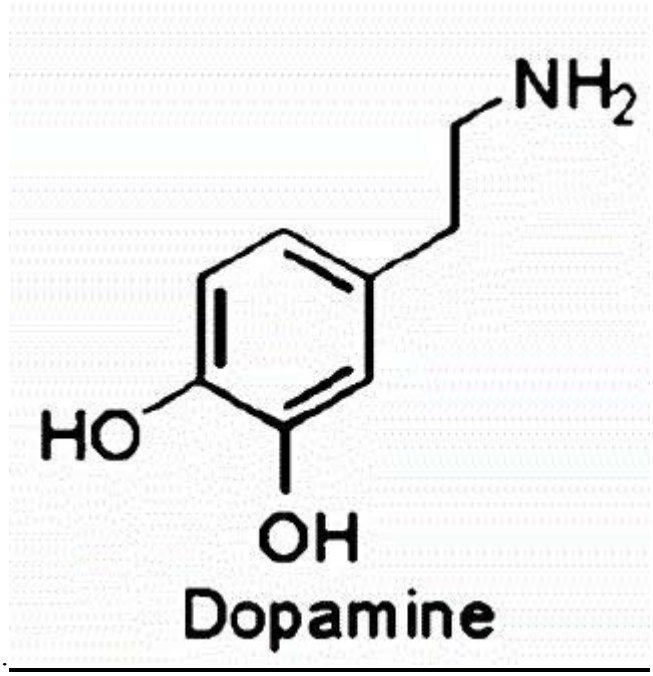

Figure 3. Chemical structure of Dopamine

The distributions of the transcripts encoding the five dopamine receptors have been determined in the human striatum and selected regions of the neocortex. In the prefrontal cortex as well as the temporal neocortex D_1 and D_4 receptor mRNAs are the most abundant, although the other three transcripts are seen at lower levels. In the occipital neocortex, D_1 receptor mRNA is the most abundant, D_3 the rarest, while the other three transcripts are present at modest levels of expression (Meador-Woodruff *et al.*, 1996).

Dopamine and spreading depression

It has been shown that SD changed neuronal activity and consequently modulated extracellular dopamine in the terminal fields during stimulation of the prefrontal cortex (Murase et al., 1993, Taber and Fibiger, 1995, Karreman and Moghaddam, 1996 and Rossetti et al., 1998). There is a pronounced release of dopamine during both spreading depression and anoxia. In spreading depression, the sharp increase of potassium concentration that follows an initial smaller and slower increase of potassium is accompanied by the release of dopamine in *in vivo* experiments (Moghaddam et al., 1987). Cortical stimulation increases basal levels of dopamine in the caudate (Strafella et al., 2001) and in the nucleus accumbens (Tucci et al., 2000; You et al., 1998). In line with these data, blockade of the prefrontal cortex activity by tetrodotoxin (Karreman and Moghaddam, 1996) or by local anesthetics (Murase et al., 1993) decreased basal dopamine levels in the nucleus accumbens. The mechanisms by which SD

can modulate the dopaminergic presynaptic terminals in striatum are unknown. There is evidence that cortex can enhance dopamine release in striatum via activation of glutamatergic neurotransmission (Cheramy et al., 1986, Cheramy et al., 1990, Kilpatrick and Phillipson, 1986, Leviel et al., 1990 and Romo et al., 1986). Other data favour an opposite view on the role of glutamate in impulse-dependent dopamine release (Wu et al., 2000 and Zhang and Sulzer, 2003) and have proposed more complicated interactions via H_2O_2 (Avshalumov et al., 2003). Another study indicates elevation of evoked dopamine release in the nucleus accumbens and a decrease in the nucleus caudatus resulting from depression of the cortical activity induced by SD. These findings suggest that in the nucleus caudatus dopaminergic presynaptic terminals are under cortical tonic activating control, but in the mesolimbic terminal fields in the nucleus accumbens, they are under tonic depression. Therefore, SD in the cortex, may modulate neurotransmitter release in subcortical structures and may have a general impact on the redistribution of the oxygen supply in these subcortical areas.

In spite of these studies, the ole of dopamine in initiation and propagation of cortical SD still needed to be clarified. The dopaminergic system has also been explored for a potential role in susceptibility to different neurological disorders. Several dopaminergic candidate genes have been investigated in different migraine case–control cohorts with varying results (Del Zompo et al.,1998; Mochi et al., 2003). Most migraine symptoms can be induced by dopaminergic stimulation. Moreover, there is dopamine receptor hypersensitivity in migraineurs, as demonstrated by the induction of yawning, nausea, vomiting, hypotension, and other symptoms of a migraine attack by dopaminergic agonists at doses that do not affect non-migraineurs. Bromocriptine, a dopamine agonist, produces a predictable dose-related series of clinical signs. Yawning is the first to appear. Increasing the dose induces mood changes, nausea, gastrokinetic changes, hypotension, vomiting, and lastly dyskinesia. Migraine patients yawned four times more often per hour and showed a higher incidence of headache than controls after 0.25 mg sublingual apomorphine, another dopamine agonist (Del Bene et al., 1994). In a large subgroup of migraineurs, dopamine acts as an endogenous protagonist in the pathophysiology of the disorder. Antagonism of this protagonist neurotransmitter therefore results in symptomatic relief of both the headache and associated symptoms (Peroutka, 1997). Prochlorperazine and Domperidone D_2 receptor antagonists have a high degree of efficacy in the acute treatment of migraine (Amery and Waelkens, 1983; Coppola et al., 1995). Neurons containing D_1 receptor may play a role in modulating trigeminovascular nociception. These neurons offer an important target to understanding pathophysiology of migraine and may offer new directions for therapy. The aim of this study was to investigate the effect of D_1 and

D_2 receptors on the characteristic features of cortical SD. Therefore, we investigated the effects of inhibition of both D_1 and D_2 receptors on SD in rat neocortical tissues.

Material and methods

The experiments were performed on adult rat (250-350g) somatosensory neocortical slices. The brain was removed under deep methohexital anaesthesia and placed in cold (1–4°C) artificial cerebrospinal fluid (ACSF) pre-equilibrated with 5% CO_2 in O_2 to give a pH of 7.4. The ACSF contained (in mM): NaCl 124, KCl 4, $CaCl_2$ 1.0, NaH_2PO_4 1.24, $MgSO_4$ 1.3, $NaHCO_3$ 26 and glucose 10. The somatosensory neocortices were dissected and cut into slices of 500 μm thickness. The slices were incubated in ACSF solution for >1 h at 28°C. After 30-min incubation, $CaCl_2$ was elevated to 2.0 mM. Slices were transferred to an interphase-type experimental chamber and superfused with ACSF at 32°C (1.5–2 ml/min).

Electrophysiological recordings

Extracellular field potentials were recorded with glass microelectrodes (150 mmol/l NaCl; 2–10 MΩ) connected to the amplifier by an Ag/AgCl–KCl bridge in the third and the fifth layers of neocortical tissues. Field potentials were traced by an ink-writer and recorded by a digital oscilloscope.

Induction of neocortical SD

SD was elicited by KCl microinjection. 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 sixth layer of the neocortical slices. A high-pressure pulse was applied to inject an amount of K^+ in the tissue sufficient to induce cortical SD (tip diameter: 2 μm; injection pressure 0.5–1.0 bar applied for 200–300 ms, two injections, 1–3 nl per pulse). Cortical SD-like events were evaluated with respect to their amplitude, duration and velocity rates. SD duration was defined as the interval between the time of half-maximal voltage shift during onset and recovery of the negative DC potential deflection.

Long-term potentiation

Single pulses of electrical stimulation were applied through a bipolar platinum electrode attached to the white matter perpendicular to the recording electrodes. Evoked field excitatory postsynaptic potentials (fEPSP) were recorded in the third layer of neocortical slices. The fEPSP was elicited by adjusting the intensity of stimulation to ~50% of that at which population spikes after fEPSP began to appear. The amplitude of fEPSP 1 ms after the onset was measured for data analysis. In long-term potentiation (LTP) experiments, the cortex was sequentially stimulated once every minute. Ten trains of four pulses (pulse duration 0.1 msec; interpulse interval 50 msec; intensity 5 V) were repeated at intervals of 10 msec. LTP was operationally defined as the mean change in fEPSP amplitude in response to five stimuli given 30 min after tetanic stimulation compared with the mean response to five test pulses applied immediately before the stimulation. Thus % potentiation = [(posttetanus amplitude of fEPSP/baseline amplitude of fEPSP) 1] 100. Tetanic stimulation was applied 60 min after application of drug.

Experimental protocols

The experimental protocol consisted of four periods as follows: (a) control period, neocortical slices were superfused with ACSF (30 min), tested for spontaneous SD; (b) KCl injection, induction of SD (SD1); (c) application of D2 dopamine receptor agonist quinpirole (10-200 μM), or the dopamine D2 dopamine receptor antagonist sulpiride (0.1-10 μM, 60 min) before the second injection of KCl (SD2); (d) washout of quinpirole or sulpiride with ASCF (45 min, second control period), third injection of KCl (SD3). Only a single concentration of quinpirole or sulpiride was used in a given slice. In control experiments, DMSO (0.5%) was added to the bath solution after the first KCl injection (60 min) and washed with ASCF (45 min) after the second and before the third KCl application.

Drugs

Quinpirole or sulpiride both purchased from Sigma-Aldrich.

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 Holm-Sidak's test. Significance was established when the probability values were less than 0.05. The investigations were approved

by the local ethics committee (Tierversuchsgenehmigung, Bezirksregierung Münster, Deutschland, AZ: 50.0835.1.0, G79/2002).

Results:

The effect of D2 dopamine receptor agonist quinpirole on SD

Focal application of KCl in the sixth layer of neocortical tissues induced negative DC deflections followed by positive waves (amplitude of 15.6 ± 1.9 mV; duration of 113 ± 5 sec). Negative DC-fluctuations were sometimes preceded by small positive waves. These cortical SD waves propagated opposite to the direction of the ACSF flow at propagation velocity of 3.1 ± 0.1 mm / min. The effect of five different concentrations of D2 dopamine receptor agonist quinpirole (10, 20, 50, 100, 200 μM; n = 6 for each concentration) was tested on potassium-evoked SD in neocortical tissues. The ratio between the second and the first DC potential waves (SD2/SD1) was calculated in control slices and slices treated with quinpirole. Sixty minutes of quinpirole application at 10 μM did not significantly change different characteristics of SD, i.e. amplitude, duration, and propagation velocity. Quinpirole at higher concentrations dose-dependently increased the amplitude and the duration of negative depolarisation potential shifts occurring after the second KCl application (SD2). The amplitude as well as the duration of SD2 and the SD2/SD1 ratio significantly increased after superfusion of quinpirole at 20-200 μM (Fig. 4; $P \leq 0.001$; ANOVA test, Holm-Sidak' method). Quinpirole increased the SD amplitude and duration between 23 ± 4 to 80 ± 7 % and between 18 ± 5 to 59 ± 5 % of the baseline level, respectively. Quinpirole did not change the velocity of negative DC potential propagation at all different concentrations. After washout of the compound, the amplitude, the duration, and the velocity of the propagation of the negative DC waves (SD3) returned close to the initial levels (SD1; Fig. 4).

The effect of dopamine D2 dopamine receptor antagonist sulpiride on neocortical SD

Sulpiride at 1-10 μM dose-dependently decreased the amplitude of negative DC potentials occurring after the second KCl application (SD2; Fig. 5; $P \leq 0.001$; ANOVA test). Application of sulpiride for sixty minutes reduced the SD amplitude to 38 ± 5 % of the baseline level (SD2/SD1 ratio). Sulpiride at these concentrations also significantly and dose-dependently decreased the mean duration of cortical SD to 48 ± 6 % of the baseline value. Sulpiride at all different concentration did not change the speed of the DC-wave propagation. Sulpiride at 0.1 μM did not affect SD. After washout of the compound, the amplitude of the deflection of DC potentials (SD3) returned close to the initial levels (SD1; Fig. 5).

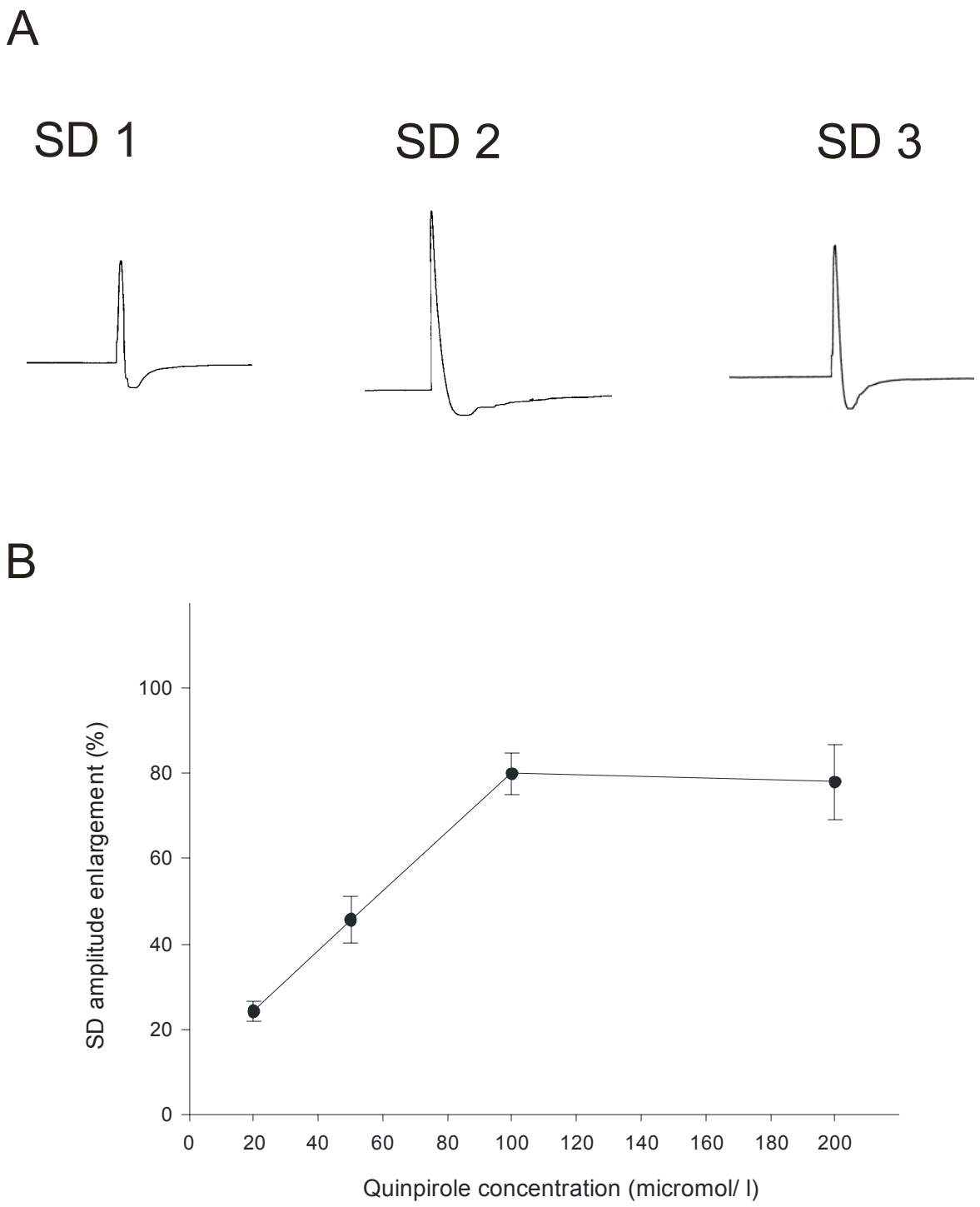

Figure 4. Effects of quinpirole on cortical spreading depression (SD) in somatosensory neocortical tissues. A: Recording of DC potential shifts in the third layer of a neocortical slice before (A1), during (A2), and after (A3) application of quinpirole (50 μM). Field potentials were recorded by an ink-writer. SD was elicited by KCl microinjection. B: The curve indicates the plot of percentage enlargement of SD amplitude vs. quinpirole concentrations (n = 6 for each concentration). Quinpirole dose-dependently increased the amplitude of SD. The

percentage of SD amplitude enlargement was measured by division of the amplitude of SD induced after application of sulpiride to the amplitude of SD elicited before superfusion of the substance. Values represent mean ± SEM. Significance was determined by ANOVA test followed by Dunn's post-test (B; $P \leq 0.001$).

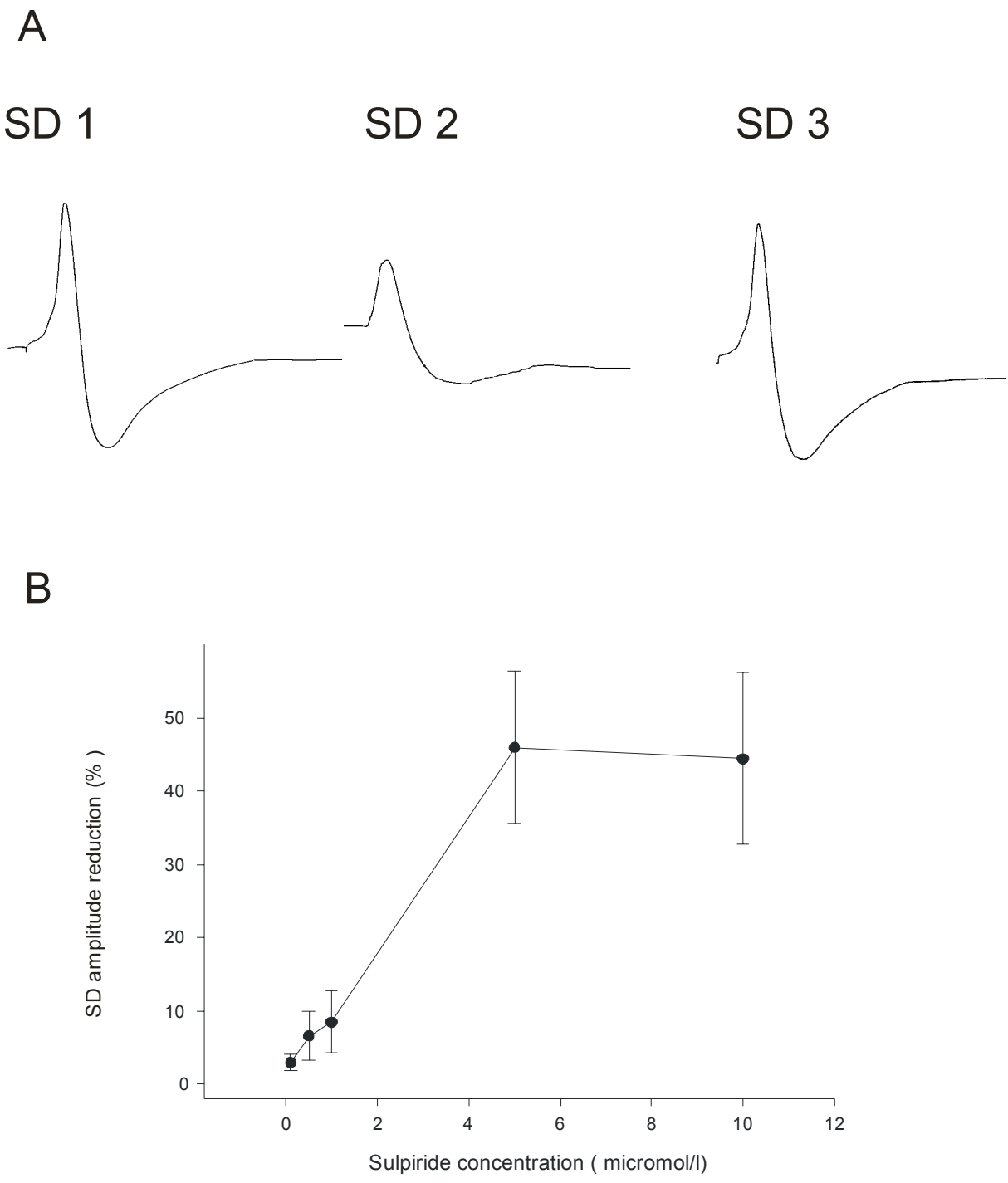

Figure 5. Effects of sulpiride on cortical spreading depression (SD) in somatosensory neocortical tissues. A: Recording of DC potential shifts in the third layer of a neocortical slice before (A1), during (A2), and after (A3) application of sulpiride (5 μM). Field potentials were

recorded by an ink-writer. SD was elicited by KCl microinjection. B: The curve indicates the plot of percentage decreases of SD amplitude vs. sulpiride concentrations (n = 6 for each concentration). Sulpiride dose-dependently decreased the amplitude of SD. The percentage of SD amplitude reduction was measured by division of the amplitude of SD induced after application of sulpiride to the amplitude of SD elicited before superfusion of the substance. Values represent mean ± SEM. Significance was determined by ANOVA test followed by Dunn's post-test (B; $P \leq 0.001$).

The effect of quinpirol and sulpiride on LTP

A conditioning tetanic stimulation was delivered to the white substance of neocortical slices followed by pulses with stimulation parameters identical to control values. The evoked fEPSP was stable for at least 30 min before application of tetanic stimulation (less than 10% variation; Fig. 6). Administration of tetanic stimulation produced a rapid and stable enhancement of the amplitude of the fEPSP in all tested preparations (n = 6, 164 ± 12 % control; Fig. 6). LTP lasted as long as the fEPSP were recorded (at least for 90 min). The potentiation rose within 1–2 min and stabilized within 5 minutes after the train of stimulations. Application of sulipride (5 μM; n = 10) sixty min before tetanic stimulation significantly suppressed LTP induction in all tested slices (122 ± 3 % baseline, Mann–Whitney Rank Sum test; $P \leq 0.001$, Fig. 6). However, Application of quinpirole (50 μM; n = 10) sixty min before tetanic stimulation did not significantly change the LTP induction in compare with control tissues (147 ± 6 % baseline, Mann–Whitney Rank Sum test; $P = 0.08$, Fig. 6).

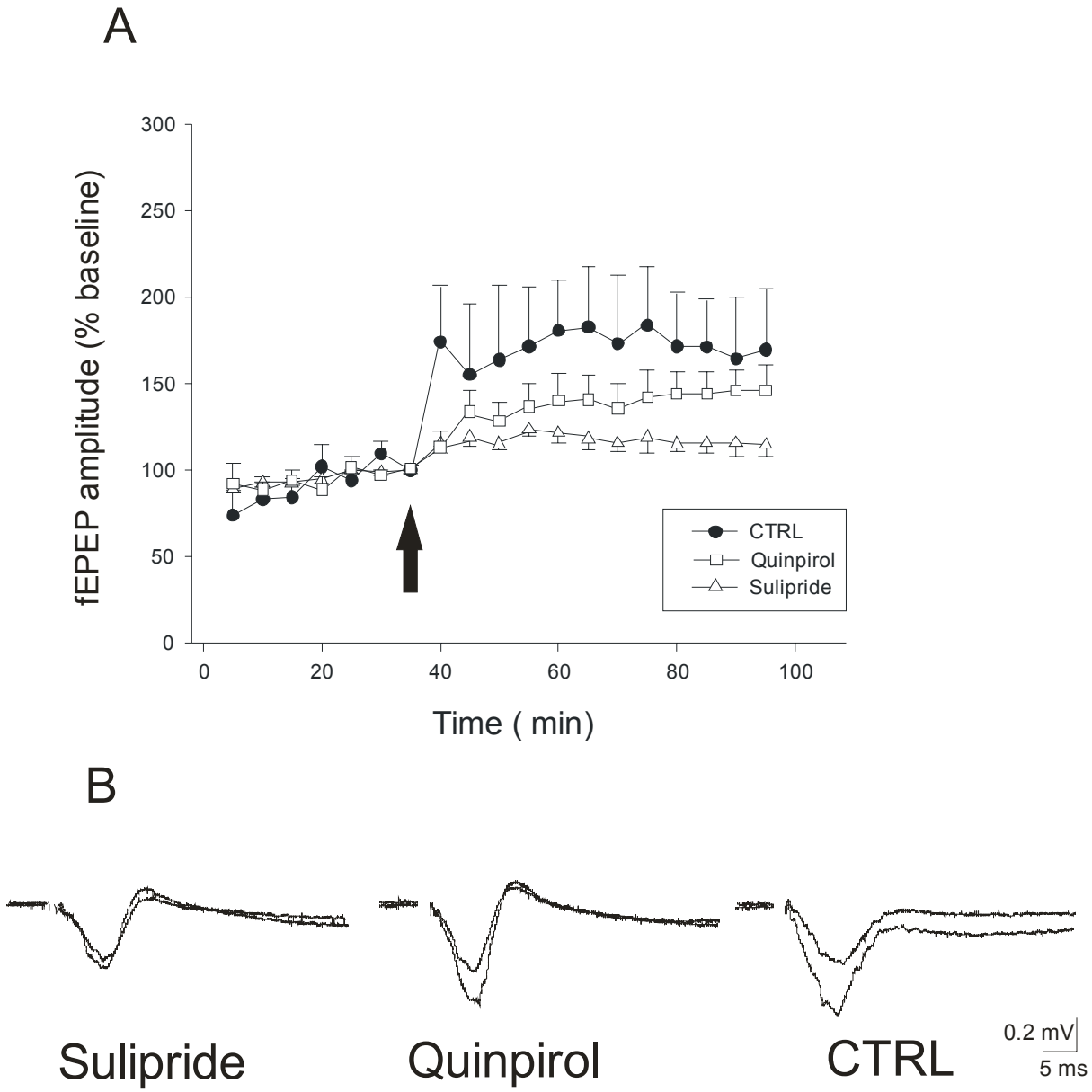

Figure 6. The effect of D2 dopamine receptor agonist quinpirole and the dopamine D2 dopamine receptor antagonist sulpiride on long-term potentiation (LTP) of the evoked field excitatory postsynaptic potentials (fEPSP) in neocortical preparations. (A) Tetanic stimulation (Ten trains of four pulses; pulse duration 0.1 msec; interpulse interval 50 msec; intensity 5 V)) produces a rapid and stable potentiation in the amplitude of the evoked field potentials, calculated as a percentage of baseline mean response amplitude. Open triangles, open square, and closed circles show the evoked fEPSP after application of sulipride (5 μmol/l), quinpirole

(50 μmol/l) and control, respectively. Arrow shows the time of tetanic stimulation, 60 min after application of substances. Application of sulipride significantly inhibited LTP of the evoked field potentials (Mann–Whitney Rank Sum test, $P = 0.001$), calculated as a percentage of baseline mean response amplitude. B: Representative examples of the evoked field potentials before and after tetanic stimulation in sulipride, quinpirole, and ACSF (control) affected slices.

Dsicussion

The present data reveal a dose dependent suppression of the amplitude and duration of the neocortical SD in the presence of the dopamine D2 dopamine receptor antagonist sulpiride. In contrary, D2 dopamine receptor agonist quinpirole dose dependently enhanced the amplitude and duration of the neocortical SD. The data point to the involvement of D2 dopamine receptor in initiation of neocortical SD. Furthermore, application of D2 dopamine receptor antagonist significantly suppressed LTP, whereas, D2 dopamine receptor agonist did not change LTP. This indicates the modulatory effect of this receptor-type on the somatosensory neocortical synaptic transmission.

Dopamine is widely distributed in the central nervous system and serves a variety of functions in the mature brain, including control of movement, cognition, endocrine responses, and reward. Dysfunction of dopaminergic system plays an important role in many neurological and psychiatric disorders, including schizophrenia, Parkinson's disease, attention-deficit hyperactivity disorder, and drug addiction (Arnsten and Li, 2005; Biederman and Faraone, 2005; Kalivas and Volkow, 2005). Dopamine receptors are G protein-coupled receptors, characterized by an extracellular N-terminal region, intracellular C-terminal region, and seven membrane-spanning regions. There are two subfamilies of DA receptors, D_1 receptors and D_2 receptors, based on their pharmacological profiles and sequence homology (Lachowicz and Sibley, 1997; Missale et al., 1998). D_1 receptors, including the D_1 and D_5 receptor subtypes, catalyze synthesis of cAMP. D_2 receptors, including the D_2, D_3, and D_4 receptor subtypes, inhibit cAMP synthesis. The receptors also affect activation of potassium channels and mitogen-activated protein kinases (Neve et al., 2004; Beaulieu et al., 2005). Several studies have identified binding partners for the D_2 receptor including coreceptors, signaling molecules, and scaf-folding proteins (Smith et al., 1999; Macey et al., 2004; Negyessy and Goldman-Rakic, 2005; So et al., 2005; Liu et al., 2006, 2007; Rashid et al., 2007; Kim et al., 2008).

The D_2 dopamine receptor has been one of the most extensively investigated gene in neurological as well as psychological disorders. A higher D_2 A_1 allelic frequency and prevalence was reported in alcoholics when compared to controls. Variants of the D_2 gene have also been associated with cocaine, nicotine and opioid dependence and obesity. The D_2

gene has also been implicated in schizophrenia, posttraumatic stress disorder, movement disorders and migraine (Noble, 2003).

A huge amount of data suggests that dopaminergic activation is a primary pathophysiologic component in certain subtypes of migraine (Peroutka, 1997). This has led to an examination of D_2 variants in this disorder. In one study the NcoI D_2 C to T polymorphism located in exon 6 was assessed in individuals having migraine with aura and without aura (Peroutka, 1997). Individuals having migraine with aura had a significantly higher frequency of the D_2 C allele than did control or migraine without aura individuals. No D_2 C allele frequency difference was found, however, between the latter two groups. The association of NcoI DRD2 variants in comorbid migraine with aura, anxiety and depression was also reported (Peroutka, 1998). The D_2 C allele frequency was significantly higher in individuals with migraine without aura, anxiety disorders or major depression than in individuals who had none of these disorders. Another group (Del Zompo et al.,1998) utilized the Transmission Disequilibrium Test and the dinucleotide repeat alleles within intron 2 of the D_2 gene to test for association with patients affected by migraine without aura. Although no difference was observed in D_2 repeat allelic distribution in the overall sample, allelic distribution differed significantly in a subgroup of dopaminergic migraineurs. Another D_2 gene polymorphism (promoter -141C Ins/Del), however, was not found to be associated with migraine (Maude et al., 2001). Furthermore, a significant and independent association was found of SNPs in the insulin receptor and the D_2 SNP93 with migraine subjects (McCarthy et al., 2001).

SD is believed to play a crucial role in migraine with aura. In 1945, Leão and Morison hypothesized that the slow march of the negative (suppressive) neurological symptoms, e.g., scotoma or hemianopia appeared after positive (stimulative) ones, e.g., flashing lights in the visual or sensory sphere is related to the SD phenomenon (Leão and Morison 1945). SD consists of a wave of neuronal activation followed by a suppression of neuronal activity that propagates slowly across the surface of the brain. SD-like waves were recorded from human neocortex during the aura phase of migraine attacks (Welch et al. 1993; Hadjikhani et al. 2001). Furthermore, increasing evidence suggesting the intense perturbations generate the cellular, molecular, and vascular changes in brain akin to SD could cause the headaches of aura-induced migraine (Moskowitz et al. 1993; Bolay et al. 2002; Gorji et al. 2004). It has been shown that sulpiride is an effective substance in the treatment of migraine headache (Piccini et al., 1990; Siniachkin et al., 1997). In the present study, sulpiride suppressed

characteristic features of SD. This suggests that blocking effect of D_2 dopamine receptor antagonist, sulpiride on neocortical SD may be responsible for its efficacy in migraine headache. Several other anti-migraine substances such as Topiramate, valproate, propranolol, amitriptyline, and methysergide also have inhibitory effects on SD (Ayata et al., 2006).

LTP is an experimental phenomenon, which can be used to demonstrate the repertoire of long-lasting modifications of which individual synapses are capable (Collingridge & Singer 1990, Malenka & Bear 2004). In the present experiments, neocortical slices perfused with sulpiride exhibited a pronounced, persisting, and significant suppression of LTP, whereas, D_2 receptors agonist, quinpirol did not change LTP. Induction of LTP in the synaptic pathway from the basolateral amygdala to the dentate gyrus is regulated by D_2 dopamine receptors (Abe et al., 2009). In line with our data, it has been reported that blocking of D_2 dopamine receptors led to the inhibition of LTP (Abe et al., 2008). It has been suggested that the role of dopamine D_2 receptors in the induction of LTP is modulatory and depends on GABAergic inhibition (Abe et al., 2009). SD induces an LTP-like effect in rat neocortical slices (Footitt and Newberry 1998) and enhances LTP induction in human neocortical tissues (Berger et al., 2008). Both inhibition of LTP induction and SD generation were observed by drug manipulation in rat neocortical tissues (Muller et al., 2006). Conversely, enhancement of LTP induction and facilitation of SD occurrence was observed under female hormones application in rat somatosensory neocortical tissues (Sachs et al, 2007). Modulation of LTP responses was also observed remote from the SD propagation site in hippocampal tissues (Wernsmann et al. 2006). The inhibition of LTP after administration of dopamine D_2 receptors and reduction of synaptic efficacy may be responsible for its suppressive effect on SD.

Conclusion

D2 dopamine receptors seem to participate in the pathophysiological mechanisms of migraine attacks as well as other neurological and psychological disorders. Inhibition of D2 dopamine receptors plays an important role in the treatment of these disorders including migraine with aura. The therapeutic effects of blocking D2 dopamine receptors in migraine attacks may be due to its inhibitory action on neocortical SD. In addition, present data indicate the importance of D2 receptors in neocortical synaptic efficacy which may be involved in its inhibitory action on SD.

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Lebenslauf

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19. Dez. 2002

Tierschutz;
Durchführung von Versuchen an Wirbeltieren

Ihr Antrag vom 10.10.2002, hier eingegangen am 06.11.2002

Genehmigungsbescheid:

Sehr geehrter Herr Professor Speckmann,

gemäß § 8 Tierschutzgesetz (TierSchG) vom 25. Mai 1998 (GBGl. I S. 1105) in der zur Ze geltenden Fassung wird Ihnen die Genehmigung zur Durchführung nachstehenden Versuchsvorhabens erteilt:

„Experimentelle Epilepsieforschung". (10 Teilprojekte gem. Antrag)

Leiter des Versuchsvorhabens und seine Stellvertreter sind:

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