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PATTERNS OF NEUROTRANSMITTER RECEPTOR DISTRIBUTIONS FOLLOWING CORTICAL SPREADING DEPRESSION

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Abstract—Spreading depression (SD), a self-propagating depolarization of neurons and glia, is believed to play a role in different neurological disorders including migraine aura and acute brain ischaemia. Initiation and propagation of SD modulate excitability of neuronal network. A brief period of excitation heralds SD which is immediately followed first by prolonged nerve cell depression and later by an excitatory phase. The aim of the present study was to characterize local and remote transmitter receptor changes after propagation of cortical SD. Quantitative receptor autoradiography was used to asses 16 transmitter receptor types in combined striatum-hippocampus–cortex slices of the rat 1 h after induction of cortical SD. In neocortical tissues, local increases of glutamate NMDA, AMPA, and kainate receptor binding sites were observed. In addition to up-regulation of ionotropic glutamate receptors, receptor binding sites of $GABA_A$, muscarinic M1 and M2, adrenergic α_1 and α_2, and serotonergic $5\text{-}HT_2$ receptors were increased in the hippocampus. Cortical SD also upregulated NMDA, AMPA, kainate, $GABA_A$, serotonergic $5\text{-}HT_2$, adrenergic α_2 and dopaminergic D1 receptor binding sites in the striatum. These findings indicate selective changes in several receptors binding sites both in cortical and subcortical regions by SD which may explain delayed excitatory phase after SD. Mapping of receptor changes by cortical SD increases our understanding of the mechanism of SD action in associated neurological disorders.

Key words: migraine headache, stroke, epilepsy, transmitter density, autoradiography.

Spreading depression (SD) belongs in the domain of the pathophysiology of the brain (Leao, 1944). It is a pronounced self-propagating depolarization of neurons and glia. SD spreads slowly from the site of onset as a radial wave across the neuronal tissue. This phenomenon is accompanied by a dramatic failure of brain ionic micromilieu and efflux of excitatory amino acids from nerve cells (Somjen, 2001). The hypothesis that migraine aura is the human equivalent of cortical SD is well established (Goadsby, 2007). SD also occurred following head injury or intracranial haemorrhage in human neocortex (Strong and Dardis, 2005). Furthermore, experimental investigations indicated that SD may also play a role in transient global amnesia, epilepsy and spinal cord disorders (Gorji, 2001; Gorji et al., 2004).

Several manifestations of SD indicate that different receptors may play important roles in this remarkable alteration of the brain. NMDA-receptor activation could well contribute to SD initiation and propagation (Smith et al., 2006). Both alpha- and beta-adrenergic receptors reversibly control the migration of SD-related DC potential shifts (Richter et al., 2005). 5-HT depletion enhances development of SD in rat neocortical tissues as well as its effect on SD-induced trigeminal nociception (Supornsilpchai et al., 2006). Further evidence points to suppression or activation of some receptors due to propagation of SD in neuronal tissues. Repetitive SD in neocortical tissues causes a selective reduction of paired pulse suppression which indicates a selective suppression of GABAergic function due to SD (Kruger et al., 1996). Several experiments also indicate the enhancement of synaptic strength after propagation of SD in different neuronal tissues, perhaps due to activation of glutamate NMDA- and AMPA-receptors (Gorji et al., 2004; Muller et al., 2006; Sachs et al., 2007).

A brief period of excitation heralds SD which is immediately followed by prolonged nerve cell depression (Somjen, 2001). However, studies revealed a late excitatory following depression phase of SD. This late excitatory period may be important in the pathophysiology of SD-related neurological disorders (Berger et al., 2008). A current investigation showed that induction of SD initially suppresses synaptic activity, which is followed by irreversible potentiation of synaptic strength in human neocortical tissues (Berger et al., 2008). In addition, altered neural circuit function can be seen remote from the SD propagation site (Bures et al., 1961; Kunkler and Kraig, 2003). Studies also demonstrate a late excitable state of neuronal activities in structures remote from cortical SD (Dehbandi et al., 2008; Gorji et al., 2004; Wernsmann et al., 2006). Propagation of SD is accompanied by the release and diffusion of several chemical mediators, such as excitatory amino acids, neurokinin, calcitonin gene-related peptide, serotonin and brain-derived neurotrophic factor into the interstitial space which may change the receptor affinities and subsequently alter the neuronal network activities (Gorji, 2001).

In spite of several studies on brief period of excitation heralds SD or transient depression accompanied by SD

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Abbreviations: ACSF, artificial cerebrospinal fluid; BDNF, brain-derived neurotrophic factor; LTP, long-term potentiation; SD, spreading depression.

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propagation, the biological mechanisms of the late excitatory period followed by SD need to be elucidated. Receptor analysis has clearly proven to be most helpful to access the potential biological and pathophysiological actions of peptide family, especially in heterogeneous tissues such as the brain (Dumont et al., 2004). The present observations were aimed to analyse transmitter receptor changes 1 h after propagation of SD in the neocortex as well as its remote effects in the hippocampus, caudate nucleus, and putamen with quantitative *in vitro* receptor autoradiography in rat brain tissues.

EXPERIMENTAL PROCEDURES

Recordings of bioelectrical activity

Slice preparation. Adult Wistar rats (250–300 g) were decapitated under deep methohexital anaesthesia and the brains were rapidly removed to ice-cold (4 °C) artificial cerebrospinal fluid (ACSF). The cerebellum was removed and a cut was made to divide the two cerebral hemispheres. Combined striatum-hippocampus–cortex slices containing the temporal cortex, the entorhinal cortex, the subiculum, the dentate gyrus, the hippocampus, as well as the striatum (500 μm) were cut in a nearly horizontal plane. Up to two different slices from each side were collected in a preparation. Slices were stored at 28 °C in ACSF, which contained (in mmol/l) NaCl, 124; KCl, 4; $CaCl_2$, 1.0; NaH_2PO_4, 1.24; $MgSO_4$, 1.3; $NaHCO_3$, 26; glucose, 10 (pH 7.4), oxygenated with 95% O_2 and 5% CO_2 for >1 h. After 30 min incubation, $CaCl_2$ 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. All experiments conformed to named local and international guidelines on the ethical use of animals. The number of animals used and their suffering were minimized in our experiments.

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 hippocampal CA1 (st. pyramidale) and the striatum as well as in the third layer of temporal and entorhinal cortices. Field potentials were traced by an ink-writer and recorded by a digital oscilloscope. The ink-writer recorded the sustained potential shifts and the oscilloscope the evoked potentials from the same microelectrode.

Induction of SD. A glass electrode filled with 2 M KCl or ACSF (control) was fixed in a special holder connected with plastic tube to a pressure injector and the tip inserted into the temporal neocortical slices (layer I–II). 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). The same amount of ACSF was applied in the slices in control experiments. 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.

Quantitative receptor autoradiography

Tissue processing and sectioning. One hour after induction of SD in neocortical part of the combined slices, the brain slices were frozen in isopentane (−50 °C), and stored at −70 °C until further studies. Each brain slice was serially sectioned at 10 μm in a cryostat microtome at −18 to −20 °C. Alternating sections were used for the different receptor binding protocols (see below).

Labelling procedure. The procedures of quantitative receptor autoradiography used in this study are described in detail elsewhere (Zilles et al., 2002a,b). In short, it consists of three steps: a pre-incubation, a main incubation, and a rinsing step.

The pre-incubation step is done to rehydrate the sections and wash out endogenous substances. The determination of specific binding site densities requires the undertaking of two parallel main incubation procedures. In one of these, the total binding of a given receptor type is visualized by incubating the sections in a solution containing a specific tritiated receptor ligand. In the other, non-specific binding was determined in adjacent sections by incubation with the tritiated ligand in the presence of an unlabelled competitor. Non-specific binding was less than 5% of total binding in all cases. Therefore, the total binding presents approximately the specific binding. Finally, the rinsing step stops the binding procedure and eliminates surplus tritiated ligand as well as buffer salts. Incubation protocols are summarized in Table 1.

Automated image and data analysis. Details of image and data analysis of autoradiographs were published elsewhere (Zilles et al., 2002a,b). In brief, the gray values of the autoradiographs were measured using an image analysis system (KS 400®, Zeiss, Germany) and a CCD camera (Sony, Tokyo, Japan). Gray values were transformed into concentrations of radioactivity by a nonlinear procedure based on measurements obtained from the co-exposed tritium-labelled plastic standards.

In order to extract numerical values for the mean regional receptor densities in an anatomically defined area, comparison with a cell-body-stained histological section is necessary. Therefore, cell-body-staining was performed on the same sections after developing the autoradiograms using cresyl-violet staining.

For the anatomical localization of different regions of interest (ROIs) in an autoradiograph, a print of the digitized autoradiograph and its cell-body-stained section are superimposed by means of a microscope equipped with a drawing tube. The cytoarchitectonic borders of CA1, CA3, hilus, and (molecular and granular layers of) dentate gyrus (from hippocampal formation) as well as a part of caudate-putamen (striatum) and of the temporal cortex were traced on prints according to standard cytoarchitectonical criteria (16,17). These prints with the borderlines of anatomical structures were placed on a digitizer linked with the computer for the data retrieval from the stored data matrix. The borders of the ROIs were manually traced on the tablet to define the region of interest, in which the concentration of radioactivity was calculated and transformed into receptor binding site densities (fmol/mg protein). Additionally, colour coding of autoradiographs were carried out solely to provide a clear visual impression of regional and laminar receptor distribution patterns of examined receptors.

Statistical analysis. All data are given as mean±SEM. The data were processed using the Mann–Whitney rank sum test. Significance was established when the probability values were less than 0.05. The experiments were approved by the Bezirksregierung Münster (Tierversuchsgenehmigung, Bezirksregierung Münster, Deutschland, AZ: 50.0835.1.0, G79/2002).

RESULTS

Electrophysiological recordings

Cortical SD induced in layer II–III of the temporal neocortex by local application of KCl in 48 slices. The pattern of SD propagation in combined striatum-hippocampus–cortex slices was analysed. Seventy-four percent of KCl induced SD propagated through temporal neocortex (amplitude of 12±1.5 mV and duration of 106±4 s) and ceased in en-