ELSEVIER

European Journal of Pharmacology 320 (1997) 103–109

ejp

Pharmacological reduction of electrophysiological diaschisis after photothrombotic ischemia in rat neocortex

Irmgard Buchkremer-Ratzmann, Otto W. Witte *

Neurologische Klinik, Heinrich-Heine-Universität, Moorenstraße 5, D-40225 Düsseldorf, Germany

Received 24 July 1996; revised 7 November 1996; accepted 8 November 1996

Abstract

Focal cerebral lesions in the rat brain induced by photothrombosis cause hyperexcitability of the surrounding brain. This can be demonstrated in brain slices taken from animals several days after lesioning, by analysis of field potential responses to paired-pulse stimulation. We now investigated whether and how these remote effects of a cortical lesion can be modified pharmacologically. Application of the NMDA receptor antagonist, MK-801 ((+)-5-methyl-10,11-dihydro-5*H*-dibnzo[*a*,*d*]cyclohepten-5,10-imine), was shown to block induction of immediate early genes and activation of astrocytes as evidenced by glial fibrillary acidic protein (GFAP) staining in the photothrombosis model. However, MK-801 did not affect the hyperexcitability that had been demonstrated by field potential recordings in brain slices. In another series of experiments, lubeluzole ((+)-(*S*)-4-(2-benzothiazolylmethylamino)-α-[(3,4-difluorophenoxy)methyl]-1-piperidineethanol), which inhibits the glutamate-activated nitric oxide pathway as evidenced by down-regulation of intracellular cyclic GMP, was given immediately after induction of the insult. This reduced hyperexcitability as investigated 7 days later. In the light of these data one can suggest that a nitric oxide-cyclic GMP-related mechanism may be responsible for functional alterations in the surround of photothrombotic brain lesions.

Keywords: Ischemia, focal; Photochemical infarction, treatment; NMDA receptor antagonist; Nitric oxide (NO)

1. Introduction

Improvement of the outcome following a cortical lesion as e.g. a stroke may be related not only to a reduction of lesion size, but also to effects of drugs on remote brain tissue. Thus it has been shown that remote depressions of brain metabolism can be related to functional deficits (Heiss and Herholz, 1994; Heiss et al., 1993). Such remote effects have been termed diaschisis (Von Monakow, 1914; reviewed by Andrews, 1991). Recently, we reported that cerebral photothrombosis in the rat brain is associated, not only with a metabolic diaschisis (Dietrich et al., 1986), but also with an electrophysiological diaschisis (Buchkremer-Ratzmann et al., 1996; Domann et al., 1993). In extended brain areas lateral to a lesion of the rat brain, a reduced inhibition could be demonstrated with paired-pulse stimuli applied extracellularly on brain slices. This hyperexcitability persisted for at least 60 days after lesion induction (Schiene et al., 1996; Domann et al., 1993). With intracellular recordings, a reduction of $GABA_A$ receptor-mediated inhibition could be demonstrated (Neumann-Haefelin et al., 1995). This was associated with a decrease of $GABA_A$ receptor binding in intact tissue (Schiene et al., 1996).

Diaschisis effects are usually attributed to anatomical deafferentiation. A possible alternative mechanism is cortical spreading depressions. Following induction of a cortical lesion, e.g. infarction, large waves of negativity amounting up to 22 mV travel across the cortex with a velocity of about 3 mm/min (McLachlan and Girvin, 1994; Hansen and Zeuthen, 1981). These are associated with a transient suppression of EEG (electroencephalogram) activity and were therefore called 'spreading depression'. Recent investigations suggest that such cortical spreading depressions are the major determinants for the growth of the lesion into the penumbra following occlusion of a brain artery (Nedergaard, 1994; Mies et al., 1991; Dirnagl et al., 1990). Cells within the surround of the lesion which receive enough oxygen to survive but not enough to function properly may be killed by the metabolic stress of cortical spreading depressions. Photothrombotic

* Corresponding author. Tel.: (49-211) 81-18977; Fax: (49-211) 81-14403.

PII S0014-2999(96)00891-6

infarction triggers cortical spreading depression in distant areas of the ipsilateral cortex up to 3 h after ischemia (Schroeter et al., 1995; Dietrich et al., 1994). Cortical spreading depression can be blocked by dizocilpine maleate (MK-801, (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine), a non-competitive NMDA receptor antagonist (Zhang et al., 1990; Foster and Wong, 1987).

Antagonism of glutamate-induced neurotoxicity also seems to be the mechanism of action of lubeluzole ((+)-(*S*)-4-(2-benzothiazolylmethylamino)-α-[(3,4-difluorophenoxy)methyl]-1-piperidineethanol, Lesage et al., 1996). Post-insult treatment with lubeluzole selectively prevents the slow rise of extracellular gluatamate in the peri-infarct zone (Scheller et al., 1995) and protects sensorimotor function after photothrombosis, whereas its *R*-isomer is inactive (De Ryck et al., 1996). On the cellular level, lubeluzole inhibits the nitric oxide-induced increase of cyclic GMP (Lesage et al., 1996) which can function as intra- and intercellular signal transduction system (Murad, 1994).

The aim of the present experiments was to examine whether MK-801 or lubeluzole influences the remote electrophysiological effects seen following induction of photothrombosis in rat neocortex. For these investigations we used the photothrombosis model introduced by Watson et al. (1985). This model has the advantage of being non-invasive and producing lesions with good reproducibility in terms of size and a sharp boundary between infarct and non-infarcted tissue.

2. Materials and methods

All animal protocols were developed in strict accordance with national law (Tierschutz Gesetz) and approved by Regierungspräsident Düsseldorf.

2.1. Induction of photothrombosis

Male Wistar (290–310 g) were anesthetized with halothane (2% during preparation and 1.5–1.7% during lesioning) in a mixture of O_2 and N_2 (1:2), a catheter was inserted in the femoral vein, and the rat was placed in a stereotaxic frame. Focal lesions were induced in the sensory area Parl (brain areas according to Paxinos and Watson, 1986) at the edge of the motor cortex (hindlimb area of cortex) and occipital cortex (area 2, lateral). For this purpose the skin above the skull was opened and an optic fiber bundle (aperture 1.5 mm) was positioned exactly 4 mm posterior to bregma and 4 mm lateral to the midline. The illumination with the optic fiber bundle mounted onto a cold light source (Schott KL 1500, Mainz, Germany) lasted 20 min. During the first minute Rose Bengal (Aldrich Chemie, Steinheim, Germany, 1.3 mg/100 g body weight) was injected through the femoral catheter. During the illumination the rectal temperature was monitored and held constant at 36.8–37.2°C. After induction of the thrombosis the catheter was removed and the wounds were sutured. The animals were placed in a warm environment for about 30 min. Thereafter they were responsive to mild noxious and acoustic stimuli and were returned to their cages with free access to ground food and water for 7 days.

2.2. Drug treatment

Eighteen animals did not get any treatment before or after photothrombosis. These animals served as lesioned controls for MK-801-treated animals. Eleven animals were treated with dizocilpine maleate (MK-801). Thirty minutes before the beginning of the illumination a 10-min intravenous infusion of 0.5 mg/kg body weight was started. Body temperature was carefully monitored. We observed irregular breathing and prolonged awakening of the MK-801-treated animals. Eight animals were treated with lubeluzole. Twenty-five minutes after the beginning of the illumination the animals received a 0.31 mg/kg body weight intravenous bolus followed by a 1 h intravenous infusion of 0.31 mg/kg per h (rectal temperature 36.8–37.2°C). R91154 ((*R*)-4-(2-benzothiazolylmethylamino)-α-[(3,4-difluorophenoxy)methyl]-1-piperidineethanol), the *R*-isomer of lubeluzole, was given to five rats after the induction of photothrombosis using the same treatment regimen as described for lubeluzole. Six animals were treated with a bolus and a 1 h infusion of the vehicle of R91154 and lubeluzole. These placebo-treated animals served as lesioned controls for the lubeluzole- and R91154-treated animals.

2.3. Electrophysiological recordings from brain slices

Electrophysiological recordings were obtained on day 7 after surgery. The rats were decapitated under ether anesthesia and the brains were rapidly removed. The brains were cut into coronal slices of 400 μm with a McIllwain Tissue Chopper. The slices were kept in an interface recording chamber at 33°C and superfused with artificial cerebrospinal fluid containing (in mM): NaCl 124, $NaHCO_3$ 26, KCl 5, $CaCl_2$ 2, $MgSO_4$ 2, NaH_2PO_4 1.25, and glucose 10, equilibrated with carbogen (95% O_2/5% CO_2) to pH 7.4. Field potentials were recorded using a glass capillary electrode placed in cortical layer II/III and the bipolar stimulation electrode was placed in layer VI beneath the recording electrode. Recordings were made in 0.5 mm steps lateral to the photothrombotic lesion, until the rhinal fissure was reached (Fig. 1). A double-pulse stimulation protocol was applied to investigate paired-pulse inhibition (pulses of 50 μs/5–40 V with 20 ms intervals). At each location the stimulation amplitude was chosen at which the field excitatory postsynaptic potential yielded a maximal response. The ratio of the amplitudes of the field potentials elicited by the second versus the first stimulus

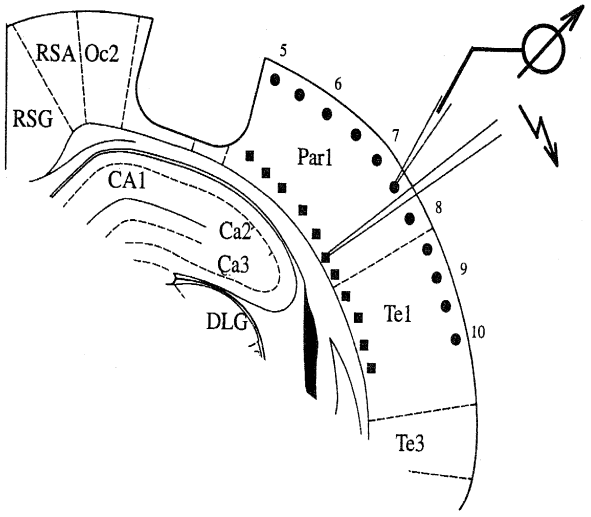

Fig. 1. Schematic drawing of the brain slice (bregma −3.8 mm; modified from Paxinos and Watson, 1986). The lesion is indicated by an indentation. Positions of the stimulation electrode are marked by squares (layer VI), those of the field potential electrode by dots (layer II/III). Numbers indicate the distance from midline in mm. Abbreviations indicate the cortical areas. Field potential and stimulation electrodes were moved in parallel from the lesion border, down to the rhinal fissure.

was calculated (Q = field excitatory postsynaptic potential$_2$/field excitatory postsynaptic potential$_1$). Values less than one indicate that the second response was inhibited by the first one. For each position, the mean, standard deviation and standard error of the mean were computed. To test the statistical significance of differences between the MK-801-treated versus untreated, lubeluzole- versus placebo-treated, and R91154- versus placebo-treated groups, respectively, we applied a non-parametric test (Kruskal-Wallis one-way analysis of variance). The significance level was set at P smaller than 0.05 ($*$), P smaller than 0.01 was considered highly significant ($**$). We examined 28 slices from 18 untreated animals; 23 slices from 11 MK-801-treated animals; 17 slices from 8 lubeluzole-treated animals; 13 slices from 5 R91154-treated animals and 9 slices from 6 placebo-treated animals.

2.4. Lesion size

Lesion size was measured from the coronal slices in the recording chamber through a reticule in the microscope. The slice which represented the center of the lesion was selected for measurement. The width of necrotic tissue at the level of layer V was measured.

3. Results

3.1. Lesion size

The infarcted area was easily detectable as necrotic tissue in the brain slices in the recording chamber. The different treatment groups were not significantly different with respect to lesion diameter: untreated 2.3 ± 0.3 mm; MK-801-treated 2.4 ± 0.2 mm; placebo-treated 2.4 ± 0.2 mm; lubeluzole-treated 2.2 ± 0.2 mm, R91154-treated 2.1 ± 0.3 mm. The infarction extended through all cortical layers but did not affect subcortical structures.

3.2. Electrophysiological recordings from brain slices

No electrophysiological responses could be evoked within the lesion. Lateral to the lesion, field potentials with amplitudes of 1–4 mV were evoked by afferent stimulation. Typical responses of control and lesioned animals, both treated and untreated, are shown in Fig. 2. Baseline recordings from control animals typically showed a Q ratio of 0.4–0.5 (Fig. 2, trace 1; Buchkremer-Ratzmann et al., 1996). In slices from untreated lesioned animals, the second field excitatory postsynaptic potential was larger than that in slices from control animals and sometimes even larger than the first field excitatory postsynaptic potential (Fig. 2, trace 2). The increase was clearly visible

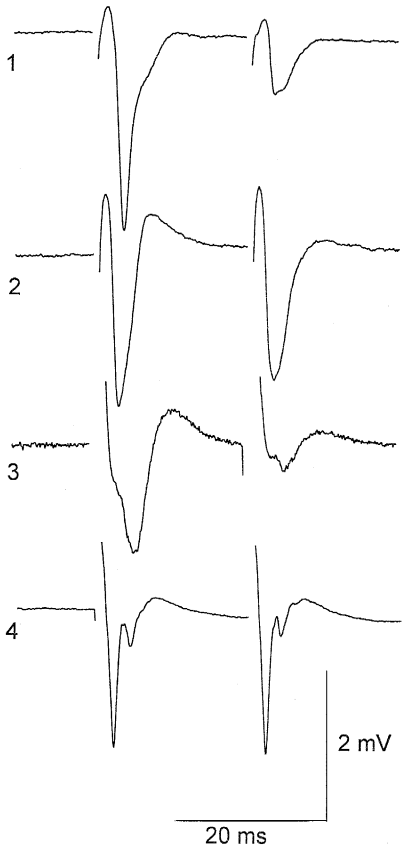

Fig. 2. Typical extracellular responses to paired-pulse stimulation. Upper trace: unlesioned control rat with a Q ratio (= field excitatory postsynaptic potential$_2$/field excitatory postsynaptic potential$_1$) of about 0.4. Second trace: untreated lesioned rat with Q near 1; third trace: lesioned rat treated with lubeluzole. Note the reduced ratio in comparison to that in an untreated rat; fourth trace: recording from lesioned rat treated with MK-801, note the lack of inhibition similar to that in untreated lesioned rat.

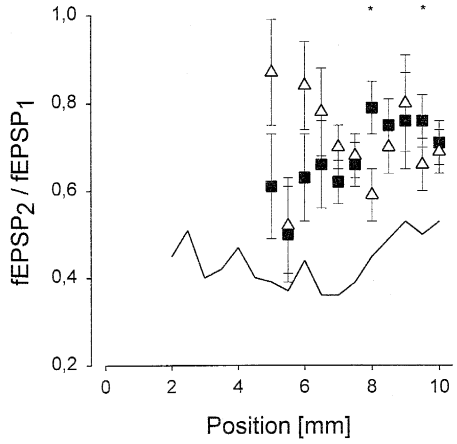

Fig. 3. Effect of MK-801 on response to paired-pulse stimuli. Spatial profile of the Q ratio (= field potential amplitudes field excitatory postsynaptic potential$_2$ /field excitatory postsynaptic potential$_1$). The lesion was situated at position 2–4.5 mm lateral from midline. Bars indicate standard error of the mean (S.E.M.). Values for unlesioned control rats are represented by the solid line, those for untreated lesioned rats are marked with black squares, MK-801-treated lesioned rats with open triangles. Mean values of recording lateral to the photothrombotic lesion. Lesioning led to an increase of the Q ratio, indicating a reduction of inhibition. MK-801 treatment did not reduce this hyperexcitability. Values for MK-801-treated rats which are significantly different from values for untreated lesioned rats are marked by $*$ for $P < 0.05$ and $**$ for $P < 0.01$ (Kruskal-Wallis, non-parametric rank test).

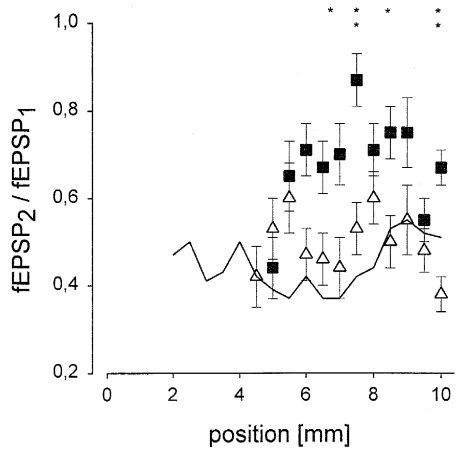

Fig. 4. Effect of lubeluzole on response to paired-pulse stimuli. Spatial profile of the Q ratio (= field potential amplitudes field excitatory postsynaptic potential$_2$ /field excitatory postsynaptic potential$_1$, mean ± S.E.M.). The lesion was situated at position 2–4.5 mm lateral from midline. Unlesioned control animals are represented by a solid line (same data as in Fig. 3), placebo-treated lesioned rats are marked with black squares, lubeluzole-treated rats with open triangles. Values for the lubeluzole group which are significantly different from values for the placebo group are marked by $*$ for $P < 0.05$ and $**$ for $P < 0.01$ (Kruskal-Wallis, non-parametric rank test). After photothrombosis a reduced inhibition can be observed in placebo-treated animals. Lubeluzole reduces the lesion-induced hyperexcitability to values similar to values from unlesioned controls.

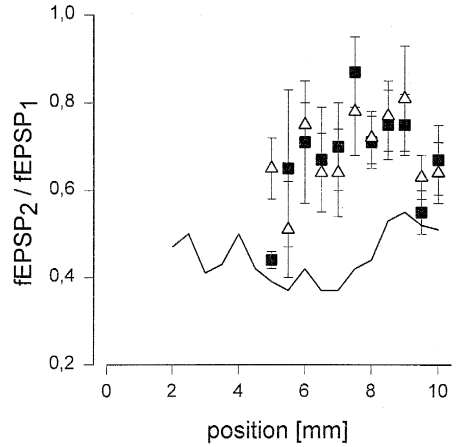

Fig. 5. Effect of the lubeluzole stereoisomer, R91154, on response to paired-pulse stimuli. Spatial profile of the Q ratio (= field potential amplitudes field excitatory postsynaptic potential$_2$ /field excitatory postsynaptic potential$_1$, mean ± S.E.M.). The lesion was situated at position 2–4.5 mm lateral from midline. Recordings from unlesioned control rats are represented by a solid line (same data as in Fig. 3Fig. 4), recordings from placebo-treated rats are marked with black squares (same data as in Fig. 4), R91154-treated rats marked with open triangles. In contrast to its isomer, R91154 did not influence the Q ratio. Values from placebo and R91154-treated animals were not significantly different (Kruskal-Wallis, nonparametric rank test).

lateral to the lesion up to 8 mm from midline (Fig. 3, black squares).

MK-801 treatment did not change the lesion-induced hyperexcitability in the surround of the lesion. The Q ratio (field excitatory postsynaptic potential$_2$/field excitatory postsynaptic potential$_1$) amounted to 0.85 near the edge of the lesion and 0.6 further lateral (Fig. 3, open triangles). With few exceptions, the values from MK-801-treated animals were not significantly different from those for untreated lesioned animals.

Treatment with lubeluzole caused a decrease of the Q ratio to values of 0.35 to 0.6 (Fig. 4) when compared to the ratio for placebo-treated animals. Placebo treatment led to results not different from the recordings from untreated lesioned animals (cf., Figs. 3 and 4/5). The data from lubeluzole-treated animals were significantly different from the data for placebo-treated animals at position 7, 7.5, 8.5, and 10 mm from midline. The differences were even more obvious when the comparison was to untreated lesioned animals, with $P < 0.01$ at positions 8.5 and 10 mm and $P < 0.05$ at positions 6, 6.5, 7, 8, 9, and 9.5. In contrast, recordings from animals treated with the isomer R91154 were not different from those for placebo-treated animals at any position (Fig. 5).

4. Discussion

Previous studies have shown that photochemically induced infarction in rat neocortex leads to focal necrosis

which is associated with a reduced inhibition in wide areas lateral to the lesion. The changes were already present on day 1 and lasted up to day 60 (Domann et al., 1993). The present work was designed to search, using pharmacological means, for a possible mechanism for the hyperexcitability remote from the lesion. We could show that the hyperexcitability induced by photochemical infarction in rat neocortex and measured by a paired-pulse stimulus protocol can be reduced by treatment with lubeluzole, but not by treatment with the NMDA receptor antagonist, MK-801.

The photothrombotic model used in the present experiments was first described by Watson et al. (1985). The strength and limitations of this model have been discussed in several reviews (Ginsberg and Busto, 1989; Hunter et al., 1995). A disadvantage of this model is that some features are not representative of human thrombotic stroke: in human stroke, main arteries rather than microvessels are occluded, that is, this model is not suitable to test therapies based on enhancement of collateral perfusion. Furthermore, unlike stroke in humans, a very early breakdown of the blood-brain barrier can be observed. However, the present model has several advantages: it is not invasive and is therefore suitable for long-term experiments. It produces lesions with a reproducible size and location. Moreover, photothrombotic lesioning leads to a sharp boundary between infarcted tissue and non-infarcted surrounding brain. It therefore allows separation of the remote effects which are not due to ischemia from those produced by ischemia in the penumbral area.

In the present experiments, cortical excitability was assessed by paired-pulse inhibition in brain slices obtained at post-infarct day 7. By that time the infarction has reached a stable volume and the brain water content does not differ at day 7 from that at 1 h after induction of the photothrombosis (Grome et al., 1988). Moreover, 3–7 days following lesion induction, the hyperexcitability in in vivo experiments was most pronounced (Schiene et al., 1996).

MK-801 is a non-competitive NMDA receptor antagonist which acts by occupying a site within the Ca^{2+} channel, acting most effectively when the channel is open (Iversen et al., 1992; Foster and Wong, 1987). This block hinders an ischemia-induced Ca^{2+} influx into the neurons. MK-801 was also shown to be an anticonvulsant with anxiolytic and central sympathomimetic properties (Clineschmidt, 1982). Systematically administered, it readily crosses the blood-brain barrier and leads to irregular breathing and arrhythmia (Choi and Rothmann, 1990; Monteau et al., 1990). MK-801 was shown to be protective in various ischemia models and studies: it was reported to antagonize glutamate toxicity in in vitro studies (Foster et al., 1988; Hahn et al., 1988; Schurr et al., 1995), and to reduce infarct volume after middle cerebral artery occlusion (Park et al., 1988; Iijima et al., 1992; Hatfield et al., 1992; Gill et al., 1992; Buchan et al., 1992; reviewed by Siesjö, 1992). However, it has also been reported that MK-801 did not reduce infarct volume after middle cerebral artery occlusion when brain temperature is held steady (Yao et al., 1993; Corbett et al., 1990). There are contradictory results of MK-801 treatment after neocortical lesioning with respect to recovery of function (Barth et al., 1994). In our model, MK-801 did not reduce infarct size as it does in other ischemia models (Dietrich et al., 1994). This could be due to the fact that, in our model, the lesion does not have a distinct penumbra, a zone of risk that can be saved by NMDA receptor blockade. Also the treatment regimen could be of importance. We used the treatment regimen described by Sae Monn Oh and Betz (1991) in which the drug is given before induction of the lesion.

Schroeter et al. (1995) and Dietrich et al. (1994) could show that photothrombotic infarction triggers cortical spreading depression. MK-801 was able to inhibit these cortical spreading depressions (Dietrich et al., 1994; Lauritzen and Hansen, 1992) and the cortical spreading depression-induced expression of GFAP (glial fibrillary acidic protein, Schroeter et al., 1995). Our results demonstrate that MK-801 treatment could not reverse the hyperexcitability surrounding a photochemical infarct. This indicates that the cortical spreading depressions are not responsible for the impairment of inhibition. These results also suggest that the hyperexcitability is not related to the activation of astrocytes as evidenced by GFAP staining.

Gass et al. (1992) showed that cerebral photothrombosis in rat cortex is associated with the activation of immediate early genes in the ipsilateral hemisphere. Activation of the immediate early genes can be prevented by application of MK-801, suggesting that it is caused by cortical spreading depressions. The present data, therefore, furthermore show that the neuronal disinhibition in the surround of the lesion is not related to cortical spreading depression-mediated activation of immediate early genes.

Huang et al. (1994) have hypothesized that activation of nitric oxide synthase is an important step in brain damage following stroke. There are ongoing discussions on the relative contribution of neuronal and vascular nitric oxide synthase activation. Activation of this synthase induces nitric oxide ($NO^{\cdot}$) formation, which inhibits mitochondrial respiration and induces ADP-ribosylation damage (reviewed by Dawson, 1994; Szatkowski and Attwell, 1994) and an increase of cyclic GMP in neurons, astrocytes and smooth muscle cells (Vincent, 1994; Nowicki et al., 1991). Cyclic GMP acts through phosphorylation or dephosphorylation of protein kinase, regulation of cAMP and in some cells (i.e. retina) through regulation of ion channels (Garthwaite, 1991). Cyclic GMP is not responsible for an increase of O_2 consumption (Lu et al., 1995). Recent reports suggest that the elevation of cyclic GMP is prevented by lubeluzole, but not by its *R*-isomer (Lesage et al., 1996). Moreover, lubeluzole reduced sensorimotor deficits after photothrombosis in rats in contrast to its *R*-isomer (De Ryck et al., 1995, 1996). The fact that the *R*-isomer fails to inhibit the $NO^{\cdot}$-induced increase of cyclic

GMP indicates that lubeluzole interacts stereoselectively with a modulation site of an enzyme or another intracellular receptor. Possible receptors could be, for example, immunophilins which interact with signal transduction systems through regulation of the phosphorylation state and catalytic activity of nitric oxide synthase (Snyder and Sabatini, 1995). Lubeluzole clearly led to a decrease of hyperexcitability in terms of the ratio of field potential amplitudes after paired pulses. Our results suggest that a nitric oxide-related mechanism might be responsible for the functional alterations in the surround of the lesion.

In conclusion, the results indicated that the electrophysiological diaschisis effects are not due to cortical spreading depression emanating from the lesion. The ineffectiveness of MK-801 as well as the bilateral occurrence of electrophysiological diaschisis would, instead, favor the assumption that it is caused by anatomical deafferentiation. Indeed, anatomical experiments indicated that widespread ipsi- and contralateral intracortical and callosal connections exist (Morin et al., 1994). More surprising is the observation that the consequences of deafferentiation can be prevented pharmacologically by lubeluzole. Further experiments are necessary to investigate whether this drug effect is related to improvement of recovery from brain infarction.

Acknowledgements

Parts of the data were presented as an abstract at Brain '95. We thank Janssen Research Foundation which kindly provided the drug, lubeluzole, and its *R*-isomer. This study is part of the doctoral thesis of I.B. Supported by DFG Wi 830/4-2.

References

Andrews, R.J., 1991, Transhemispheric diaschisis, Stroke 22, 943.

Barth, T.M., M.L. Grant and T. Schallert, 1994, Effects of MK-801 on recovery from sensorimotor cortex lesions, Stroke 21 (Suppl. 3), 153.

Buchan, A.M., A. Slivka and D. Xue, 1992, The effect of the NMDA receptor antagonist MK-801 on cerebral blood flow and infarct volume in experimental focal stroke, Brain Res. 574, 171.

Buchkremer-Ratzmann, I., M. August, G. Hagemann and O.W. Witte, 1996, Electrophyiological transcortical diaschisis after cortical thrombosis in the rat brain, Stroke 27, 1105.

Choi, D.W. and S.M. Rothmann, 1990, The role of glutamate neurotoxicity in hypoxic-ischemic neuronal death, Annu. Rev. Neurosci. 13, 171.

Clineschmidt, B.V., 1982, Effect of the benzodiazepine receptor antagonist Ro 15-1788 on the anticonvulsant and anticonflict actions of MK-801, Eur. J. Pharmacol. 84, 119.

Corbett, D., S. Evans, C. Thomas, D. Wang and A.D. Jonas, 1990, MK-801 reduced cerebral ischemic injury by inducing hypothermia, Brain Res. 514, 300.

Dawson, D.A., 1994, Nitric oxide and focal cerebral ischemia: multiplicity of actions and diverse outcome, Cerebrovasc. Brain Metab. Rev. 6, 299.

De Ryck, M., J. De Keyser, A.S. Lesage, L. Hantson and W. Swijzen, 1995, Lubeluzole reduces mortality and improves functional oucome in the treatment of acute ischemic stroke: early preclinical and clinical findings, in: Ischemic Stroke. Recent Advances in Understanding Therapy, eds. J. Grotta, L.P. Miller and A.M. Buchan (International Business Communications, Southborough, MA) p. 271.

De Ryck, M., R. Keersmaekers, H. Duytschaever, C. Claes, G. Clincke, M. Janssen and G. Van Reet, 1996, Lubeluzole protects sensorimotor function and reduces infarct size in a photochemical stroke model in rats, J. Pharmacol. Exp. Ther. (in press).

Dietrich, W.D., M.D. Ginsberg, R. Busto and B.D. Watson, 1986, Photochemical induced cortical infarction in the rat. 2. Acute and subacute alterations in local glucose utilisation, J. Cereb. Blood Flow Metab. 6, 195.

Dietrich, W.D., Z.-C. Feng, H. Leistra, B.D. Watson and M. Rosenthal, 1994, Photothrombotic infarction triggers multiple episodes of cortical spreading depression in distant brain regions, J. Cereb. Blood Flow Metab. 14, 20.

Dirnagl, U., J. Tanabe and W. Pulsinelli, 1990, Pre- and post-treatment with MK-801 but not pretreatment alone reduces neocortical damage after focal cerebral ischemia in the rat, Brain Res. 527, 62.

Domann, R., G. Hagemann, M. Kraemer, H.-J. Freund and O.W. Witte, 1993, Electrophysiological changes in the surrounding brain tissue of photochemically induced corical infarcts in the rat, Neurosci. Lett. 155, 69.

Foster, A.C. and E.H.F. Wong, 1987, The novel anticonvulsant MK-801 binds to the activated state of the *N*-methyl-D-aspartate receptor in rat brain, Br. J. Pharmacol. 91, 403.

Foster, A.C., R. Gill and G.N. Woodruff, 1988, Neuroprotective effects of MK-801 in vivo: selectivity and evidence for delayed degeneration mediated by NMDA receptor activation, J. Neurosci. 8, 4745.

Garthwaite, J., 1991, Glutamate, nitric oxide and cell-cell signaling in the nervous system, Trends Neurosci. 14, 60.

Gass, P., M. Spranger, T. Herdegen, R. Bravo, P. Köck, W. Hacke and M. Kiessling, 1992, Induction of FOS and JUN proteins after focal ischemia in the rat: differential effect of the *N*-methyl-D-aspartate receptor antagonist MK-801, Acta Neuropathol. (Berlin) 84, 545.

Gill, R., P. Andine, L. Hillered, L. Persson and H. Hagberg, 1992, The effect of MK-801 on cortical spreading depression in the penumbral zone following focal ischaemia in the rat, J. Cereb. Blood Flow Metab. 12, 371.

Ginsberg, M.D. and R. Busto, 1989, Rodent models of cerebral ischemia, Stroke 20, 1627.

Grome, J.J., G. Gojowczky, W. Hofmann and D.I. Graham, 1988, Quantitation of photochemically induced focal cerebral ischemia in the rat, J. Cereb. Blood Flow Metab. 8, 89.

Hahn, J.S., E. Aizenman and S.A. Lipton, 1988, Central mammalian neurons normally resistant to glutamate toxicity are made sensitive by elevated extracellular Ca^{2+}: toxicity is blocked by the *N*-methyl-D-aspartate antagonist MK-801, Proc. Natl. Acad. Sci. USA 85, 6556.

Hansen, A.J. and T. Zeuthen, 1981, Extracellular ion concentrations during spreading depression and ischemia in the rat brain cortex, Acta Physiol. Scand. 113, 437.

Hatfield, R.H., R. Gill and C. Brazell, 1992, The dose-response relationship and therapeutic window for dizocilpine (MK-801) in a rat focal ischemia model, Eur. J. Pharmacol. 216, 1.

Heiss, W.D., H.G. Emunds and K. Herholz, 1993, Cerebral glucose metabolism as a predictor of rehabilitation after ischemic stroke, Stroke 24, 1784.

Heiss, W.D. and K. Herholz, 1994, Assessment of pathophysiology of stroke by positron emission tomography, Eur. J. Nucl. Med. 21, 455.

Huang, Z., P.L. Huang, N. Panahian, T. Dalkara, M.C. Fishman and M.A. Moskowitz, 1994, Effects of cerebral ischemia in mice deficient in neuronal nitric oxide synthase, Science 265, 1883.

Hunter, A.J., A.R. Green and A.J. Cross, 1995, Animals models of acute ischaemic stroke: can they predict clinically successful neuroprotective drugs?, Trends Pharmacol. Sci. 16, 123.

Iijima, T., G. Mies and K.-A. Hossmann, 1992, Repeated negative DC deflections in rat cortex following middle cerebral artery occlusion are abolished by MK-801: effect on volume of ischemic injury, J. Cereb. Blood Flow Metab. 12, 727.

Iversen, L.L., G.N. Woodruff, J.A. Kemp, A.C. Foster, R. Gill and E.H.F. Wong, 1992, Neuroprotective properties of the glutamate anatagonist dizocilpine maleate (MK-801) in animal models of focal and global ischamia, in: Ischaemia, ed. W. Kriegelsteiner (Wiss. Verlag Ges.) p. 165.

Lauritzen, M. and A.J. Hansen, 1992, The effect of glutamate receptor blockade on anoxic depolarization and cortical spreading depression, J. Cereb. Blood Flow Metab. 12, 223.

Lesage, A.S., L. Peeters and J.E. Leysen, 1996, Lubeluzole, a novel long-term neuroprotectant, inhibits the glutamate-activated nitric oxide synthase pathway, J. Pharmacol. Exp. Ther. (in press).

Lu, X., H.M. Wei, J. Yospin and H.J. Weiss, 1995, Effect of nitroprusside on regional cerebral cyclic GMP, blood flow and O_2 consumption in rat, Brain Res. 669, 35.

McLachlan, R.S. and J.P. Girvin, 1994, Spreading depression of Leao in rodent and human brain, Brain Res. 666, 133.

Mies, G., S. Ishimaru, Y. Xie, K. Seo and K.-A. Hossmann, 1991, Ischemic thresholds of cerebral protein synthesis and energy state following middle cerebral artery occlusion in rat, J. Cereb. Blood Flow Metab. 11, 753.

Monteau, R., P. Gauthier, P. Rega and G. Hilaire, 1990, Effects of *N*-methyl-D-aspartate (NMDA) antagonist MK-801 on breathing pattern in rats, Neurosci. Lett. 109, 134.

Morin, C.L., S. Dolina, R.T. Robertson and C.E. Ribak, 1994, An inbred epilepsy-prone substrain of BALB/c mice shows absence of the corpus callosum, and abnormal projection to the basal forebrain, and bilateral projections to the thalamus, Cereb. Cortex 4, 119.

Murad, F., 1994, The role of nitric oxide in modulating guanylyl cyclase, Neurotransmissions 10, 1.

Nedergaard, M., 1994, Neuronal injury in the infarct border: a neuropathological study in the rat, Acta Neuropathol. (Berlin) 73, 267.

Neumann-Haefelin, T., G. Hagemann and O.W. Witte, 1995, Cellular correlates of neuronal hyperexcitability in the vicinity of photochemically induced cortical infarcts in rats in vitro, Neurosci. Lett. 193, 101.

Nowicki, J.P., D. Duval, H. Poignet and B. Scatton, 1991, Nitric oxide mediates neuronal death after focal cerebral ischemia in the mouse, Eur. J. Pharmacol. 204, 339.

Park, C.K., D.G. Nehls, D.I. Graham, G.M. Teasdale and J. McCulloch, 1988, The glutamate antagonist MK-801 reduces focal ischemic brain damage in the rat, Ann. Neurol. 24, 543.

Paxinos, G. and C. Watson, 1986, The Rat Brain in Stereotaxic Coordinates (Academic Press, London).

Sae Monn Oh and A.L. Betz, 1991, Interaction between free radicals and exitatory amino acids in the formation of ischemic brain edema in rats, Stroke 22, 915.

Scheller, D., J. Kolb, S. Szathmary, E. Zacharias, M. De Ryck, J. Van Reempts, G. Clincke and F. Tegtmeier, 1995, Extracellular changes of glutamate in the peri-infarct zone. Effect of lubeluzole, J. Cereb. Blood Flow Metab. 15, 379 (Abstract).

Schiene, K., C. Bruehl, K. Zilles, M. Qü, R. Domann, M. Kraemer and O.W. Witte, 1996, Neuronal hyperexcitability and reduction of GABA receptor expression in the surround of cerebral thrombosis, J. Cereb. Blood Flow Metab. 16, 906.

Schroeter, M., K. Schiene, M. Kraemer, G. Hagemann, H. Weigel, U.T. Eysel, O.W. Witte and G. Stoll, 1995, Astroglial responses in photochemically induced focal ischemia of the rat cortex, Exp. Brain Res. 106, 1.

Schurr, A., R.S. Payne and B.M. Rigor, 1995, Protection by MK-801 against hypoxia-, excitotoxin-, and depolarization-induced neuronal damage in vitro, Neurochem. Int. 26, 519.

Siesjö, B.K., 1992, Pathophysiology and treatment of focal cerebral ischemia. Part II: Mechanisms of damage and treatment, J. Neurosurg. 77, 337.

Snyder, S.H. and D.M. Sabatini, 1995, Immunophilines and the nervous system, Nature Med. 1, 32.

Szatkowski, M. and D. Attwell, 1994, Triggering and execution of neuronal death in brain ischaemia: two phases of glutamate release by different mechanisms, Trends Neurosci. 17, 359.

Vincent, S.R., 1994, Nitric oxide: a radical neurotransmitter in the central nervous system, Prog. Neurobiol. 42, 129.

Von Monakow, C., 1914, Die Lokalisation im Großhirn und der Abbau der Funktion durch kortikale Herde (J.F. Bergman, Wiesbaden) p. 26.

Watson, B.D., W.D. Dietrich, R. Busto, M.S. Wachtel and M.D. Ginsberg, 1985, Induction of reproducible brain infarctions by photochemically initiated thrombosis, Ann. Neurol. 17, 497.

Yao, H., M.D. Ginsberg, B.D. Watson, R. Prado, W.D. Dietrich, S. Kraydieh and R. Busto, 1993, Failure of MK-801 to reduce infarct volume in thrombotic middle cerebral artery occlusion in rats, Stroke 24, 864.

Zhang, E.T., A.J. Hansen, T. Wieloch and M. Lauritzen, 1990, Influence of MK-801 on brain extracellular calcium and potassium activities in severe hypoglycemia, J. Cereb. Blood Flow Metab. 10, 136.