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Lubeluzole blocks increases in extracellular glutamate and taurine in the peri-infarct zone in rats

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Abstract

A microdialysis probe was positioned inside the peri-infarct zone of a photochemically induced neocortical infarct in rats. Extracellular glutamate rose within 20 min after the start of infarct induction and continued to increase during the 5 h observation period to 5.5-fold the pre-infarct baseline value of 0.8 ± 0.4 μmol/l. Glutamine increased only 1.4-fold. Changes in peri-infarct glutamate were preceded by steep rises in taurine (a 3.9-fold increase from the baseline value of 2.8 ± 0.7 μmol/l), which coincided with spreading depressions during infarct induction. Post-treatment with lubeluzole ((*S*)-4-(2-benzothiazolylmethylamino)-α-[(3,4-difluoro-phenoxy)methyl]-1-piperidineethanol, 1.25 mg/kg i.v.), a new cerebroprotective drug, blocked the peri-infarct increases of glutamate and taurine, whereas the *R*-enantiomer was ineffective. Since lubeluzole has previously been shown to stereospecifically decrease glutamate-activated nitric oxide (NO) toxicity in vitro, the present in vivo stereospecific effect of lubeluzole may be related to modulation of the cascade of NO toxicity, thus preventing NO toxicity-mediated increases in extracellular glutamate. Blockade of the peri-infarct taurine response suggests that lubeluzole also may have reduced cellular osmotic stress in the peri-infarct zone. © 1997 Elsevier Science B.V.

Keywords: Penumbra; Glutamate; Taurine; Lubeluzole; Neuroprotection; Swelling; Microdialysis

1. Introduction

Lubeluzole ((*S*)-4-(2-benzothiazolylmethylamino)-α-[(3,4-difluorophenoxy)methyl]-1-piperidineethanol) is a novel neuroprotective compound aimed at the treatment of acute stroke (Diener et al., 1996; De Ryck et al., 1995a, 1996). In a thrombotic stroke model in rats, intravenous post-treatment with lubeluzole rescued sensorimotor function (tactile/proprioceptive limb placing reactions) within a time-window of 6 h and reduced infarct size (De Ryck et al., 1996). Both effects were stereospecific, in that only lubeluzole was active, whereas the *R*-enantiomer of lubeluzole was inactive. Lubeluzole, administered as a sequence of intravenous and intraperitoneal injections, reduced infarct size by 50% in a middle cerebral artery occlusion model in rats (Aronowski et al., 1996). In the peri-infarct zone surrounding a thrombotic neocortical infarct in rats, lubeluzole, but not the *R*-enantiomer, normalized neuronal excitability, as evidenced by normalization of paired-pulse (γ-amino butyric acid (GABA)-mediated) inhibition, which is abolished in the peri-infarct zone (Buchkremer-Ratzmann and Witte, 1997; Domann et al., 1993). The different in vivo neuroprotective effects of lubeluzole and of its *R*-enantiomer correspond to the different potencies with which they inhibit glutamate-activated cyclic GMP production in rat embryonic neuronal cultures (Lesage et al., 1995, 1996), a consequence of glutamate-activated nitric oxide (NO) production via NO synthase. When added as prolonged pretreatment to neuronal cultures, lubeluzole was about 10 times more potent than the *R*-enantiomer to down-regulate the glutamate-activated NO synthase pathway (Lesage et al., 1996). Furthermore, when administered as post-treatment, only lubeluzole, but not the *R*-enantiomer, blocked the neurotoxicity induced by NO donors (Maiese et al., 1997). The latter in vitro neuroprotective effect had a time-window of 4–6 h, i.e., similar to the time-window for in vivo neuroprotection by lubeluzole (De Ryck et al., 1996).

The present work was based on the observation that

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paired-pulse inhibition is markedly impaired in the vicinity of a thrombotic neocortical infarct in rats (Buchkremer-Ratzmann and Witte, 1997). Our experiments were aimed at testing whether such an effect is due to enhanced excitatory neurotransmission by glutamate, well-known for its excitotoxic effects in ischemic neuronal damage (Choi and Rothman, 1990). To this end, we placed a microdialysis probe (Benveniste et al., 1984; Ungerstedt, 1984) within the peri-infarct zone of a thrombotic infarct in order to monitor the extracellular changes in glutamate and to determine the possible effects of lubeluzole and the *R*-enantiomer. We chose the model of a Rose Bengal-induced neocortical infarct in rats (Watson et al., 1985) because it allows for reproducible infarct size and locus, enabling reliable positioning of a microdialysis probe and the continuous measurement of changes in the extracellular glutamate concentration from the very start of infarct induction. In addition to glutamate, glutamine was measured because it is the supposed metabolic precursor of glutamate (Erecinska and Silver, 1990). Finally, taurine was measured because, in addition to its role as a putative inhibitory transmitter (Wheler et al., 1979; Pasantes-Morales et al., 1982), it is also released nonvesicularly for volume regulation in response to osmotic imbalances (Kimelberg et al., 1990; Pasantes-Morales et al., 1994).

A single dose of lubeluzole, which has been shown to fully rescue sensorimotor function (De Ryck et al., 1996), was administered after infarct induction. The same dose of the *R*-enantiomer was used in a separate series of experiments.

2. Materials and methods

2.1. Surgical procedures

The experiments were conducted according to the recommendations of the Declarations of Helsinki and Tokyo and to the Guidelines for the Use of Experimental Animals of the European Community. The experimental protocol was approved by the local authorities and the local ethical committee.

Male Wistar rats (250 g, anesthetized with urethane 1.55–1.95 g/kg) were fixed in a stereotaxic frame. Body temperature was maintained at 37°C with a water jacket. A burr hole of 3 mm diameter was drilled through the skull 1.5 mm posterior to and 1.5 mm lateral from the bregma, and an incision was made in the dura mater. An optic fiber (outer diameter 5 mm; Dolan–Jenner) was positioned anterior to the burr hole. A microdialysis probe (2 mm length, 0.5 mm outer diameter, Carnegie Medicin, Stockholm, Sweden; perfusate: artificial cerebrospinal fluid (aCSF); flow rate: 2 μl/min; fraction volume: 20 μl) was implanted into the supposed peri-infarct area at an angle of about 30° in the sagittal plane. The direct current (DC) signal was recorded with a microelectrode positioned at a distance of about 50–100 μm from the probe and at a depth of 1 mm. To identify spreading depressions, a second microelectrode was positioned at a defined, but variable distance from the micro-electrode positioned near the microdialysis probe. Alternatively, a laser–Doppler device was positioned instead of the second microelectrode in order to determine the spreading depression-related local cerebral blood flow transient. After 90 min of equilibration, Rose Bengal was given i.v. (70 mg/kg) and the light (30–35 kLux) was turned on for 5 min. Ten minutes later, the rats received a 10 min intravenous infusion of lubeluzole (1.25 mg/kg), of the *R*-enantiomer R091154 (1.25 mg/kg), or of equal volumes of vehicle (10% β-cyclodextrin). At 30 min before the end of the 5 h experiment, an intravenous bolus of Evans blue was administered (1 ml, 10 mg/ml in 0.9% NaCl). Thirty minutes later, asphyxia was induced and the microdialysis probe was fixed with dental cement. The brains were removed and fixed in 4% formalin with the probe inserted. The brains remained immersed for several weeks in the same fixative. Vibratome sections (100 μM) were stained with azure–eosin for histological evaluation (Fig. 4).

2.2. Physiologic variables

Mean arterial blood pressure was recorded, using a Statham pressure transducer (Statham, Oxnard, CA, USA) connected to the femoral artery. Heart rate was monitored from the ECG recorded with two electrodes fixed subcutaneously to the left fore paw and the right hind paw. The values for both parameters were analyzed and stored continuously in a computer, using in-house developed software. Blood gases in arterial blood samples taken at the beginning and at the end of the experiments were analyzed with an Eschweiler System 2000 (Eschweiler, Kiel, Germany). In order not to withdraw too much blood, further samples were taken only very occasionally.

2.3. Amino acid analysis

Amino acids were determined using high performance liquid chromatography (HPLC) with fluorescence detection after automated precolumn derivatisation with *o*-phthaldialdehyde (HPLC column: 60 × 4 mm Nucleosil C_{18}, particle size 5 μm; mobile phase A: 87.5% 0.1 mol/l Na–acetate buffer, pH 6.72, 10% methanol, 2.5% tetrahydrofurane; mobile phase B: 100% methanol; linear step gradient (min, A/B: 0, 100/0; 2, 100/0; 8, 90/10; 12, 80/20; 13, 50/50; 14, 0/100; 15, 100/0); flow rate 1.2 ml/min) (Ungerstedt, 1984).

2.4. Statistics

There were two experiments. In experiment 1, lubeluzole-treated rats were compared to vehicle-treated rats (control group 1). In experiment 2, rats treated with the

R-enantiomer were compared to vehicle-treated rats (control group 2). Statistics were performed using an analysis of variance for repeated measures (ANOVA-RM) ('split-plot model') based on SAS algorithms. Unless mentioned otherwise, the data are reported as means ± standard deviation (S.D.). Statistical significance was set at a two-tailed *P*-value of less than 0.05.

3. Results

3.1. Physiological variables

Heart rate and arterial blood pressure were monitored continuously during the course of an experiment. Blood status (pH, p_{CO_2}, p_{O_2}) was recorded at the beginning and at the end of the experiment. As evident from Tables 1 and 2 neither lubeluzole nor the *R*-enantiomer affected these parameters.

3.2. Histology

The lesion parameters were aimed at a well-delineated infarct of about 4 mm in diameter in the right frontal neocortex. The morphological characteristics of the photochemical infarcts were comparable to those reported elsewhere (Van Reempts and Borgers, 1994). In the infarct core, the vessels were congested and the neurons coagulated. The peri-infarct zone was characterized by reduced staining properties due to the presence of edematous swelling. The vessels remained open (Fig. 4).

Our results were obtained from rats whose microdialysis probes were located in the weakly stained zone directly adjacent to the edge of the infarct, as could be verified microscopically. Histological evaluation of probe position was performed in a blinded manner.

3.3. Spreading depressions

Spreading depressions were identified as waves of negative DC deflections propagating at a velocity of 3–5 mm/min along the cerebral cortex (Hansen, 1985; Lehmenkühler, 1990; Nicholson, 1993). The shape of the deflection clearly allowed a spreading depression to be distinguished from an ischemic/anoxic DC deflection (Mies et al., 1994; De Ryck et al., 1995b; Ashton et al., 1997). In addition, any spreading depression in urethane-anesthetized animals is accompanied by a transient increase of the local cerebral blood flow whereas a reduction of the local cerebral blood flow occurs under ischemic conditions with ischemic/anoxic depolarization (Kocher, 1990).

In experiment 1, spreading depressions in vehicle-treated rats ($n = 8$) appeared with a latency of 123 ± 12 s after the start of illumination. In lubeluzole-treated rats ($n = 9$), the latency was 148 ± 69 s. There were 14 ± 13 spreading depressions (range: 3–37) in vehicle-treated rats and 12 ± 11 (range: 3–33) in lubeluzole-treated rats. None of these differences was statistically significant. In experiment 2, spreading depressions in vehicle-treated rats ($n = 6$) appeared with a latency of 112 ± 26 s after the start of illumination. In rats treated with the *R*-enantiomer ($n = 9$), the latency was 117 ± 46 s. As in experiment 1, spreading depressions in experiment 2 were present for as long as 2 h after infarct induction. In experiment 2, the number of spreading depressions in control rats versus rats treated with the *R*-enantiomer was 11 ± 15 (range: 3–49) versus 5 ± 5 (range: 2–17), respectively. The differences were not statistically significant.

3.4. Glutamate

Extracellular glutamate started to rise between 20 to 30 min after the start of illumination (Fig. 1a and b).

Table 1
Heart rate (HR (bpm)) and mean arterial blood pressure (MABP, (mmHg)) in anesthetized rats with a photochemically induced unilateral neocortical infarct before and after treatment with lubeluzole or *R*-enantiomer (1.25 mg/kg i.v. at $t = 0$ min)

Time (min)	Heart rate						Arterial blood pressure					
	Control		Lubeluzole		Enantiomer		Control		Lubeluzole		Enantiomer	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
−30.00	408	58	392	14	428	22	104	5	96	10	100	9
−2.50	401	54	399	23	425	21	102	7	99	8	100	9
30.00	380	58	396	33	408	22	118	7	119	8	121	11
60.00	400	59	420	32	427	24	110	10	114	3	116	10
90.00	405	56	433	31	442	30	108	9	108	8	112	9
120.00	412	57	439	35	444	22	109	11	108	7	108	7
150.00	413	57	440	31	443	24	110	12	106	8	106	4
180.00	419	59	440	24	444	27	109	10	106	6	106	5
240.00	433	47	438	30	455	27	110	13	108	6	108	8
275.00	439	56	444	21	456	24	109	13	108	4	108	9

Table 2
-lood parameters 30 min before and 240 min after treatment (p_{O_2}, p_{CO_2} in mmHg)

		Control		Lubeluzole		*R*-Enantiomer	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
p_{O_2}	before	161	36	176	39	142	14
p_{O_2}	end	143	24	136	44	135	9
p_{CO_2}	before	47	10	50	5	44	4
p_{CO_2}	end	51	9	52	8	50	6
pH	before	7.32	0.04	7.31	0.04	7.32	0.03
pH	end	7.29	0.04	7.28	0.05	7.28	0.08

Results from experiment 1 are shown in Fig. 1a. In the vehicle-treated control group 1, glutamate rose from 0.84 $\pm$ 0.41 μmol/l before the start of illumination to 4.58 $\pm$ 1.77 μmol/l at 5 h after the start. Post-treatment with lubeluzole blocked the rise in glutamate (basal level: 0.87 $\pm$ 0.39 μmol/l; final level: 1.12 $\pm$ 0.69 μmol/l). The

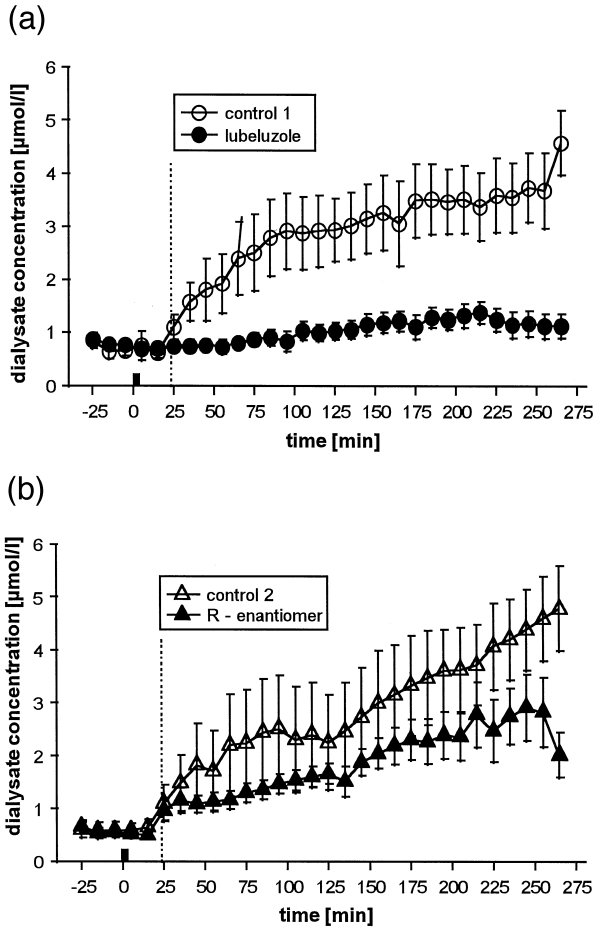

Fig. 1. Dialysate concentrations of glutamate before and after photochemical induction of a neocortical infarct in rats. Start of illumination at $t = 0$. Duration of illumination as indicated by black rectangle (■). Injection of i.v. bolus at 10 min after stop of illumination (dotted vertical line). Data are means ± S.E.M. (a) Effect of lubeluzole (●, 1.25 mg/kg i.v.) versus vehicle (○). (b) Effect of *R*-enantiomer (▲, 1.25 mg/kg i.v.) versus vehicle (△).

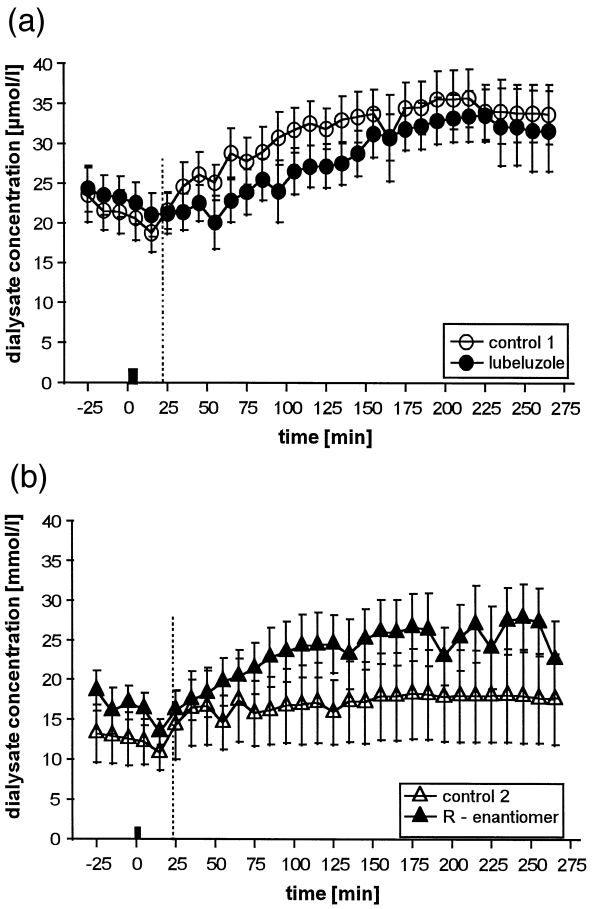

Fig. 2. Dialysate concentrations of glutamine before and after photochemical induction of a neocortical infarct in rats. Infarct induction and treatment regimen as in Fig. 1. Data are means ± S.E.M. (a) Effect of lubeluzole (●) versus vehicle (○). (b) Effect of *R*-enantiomer (▲) versus vehicle (△).

difference between the vehicle- and lubeluzole-treated groups was highly significant ($P = 0.007$).

Results from experiment 2 are shown in Fig. 1b. In the vehicle-treated control group 2, glutamate rose from 0.60 $\pm$ 0.36 to 4.78 $\pm$ 2.00 μmol/l. Following post-treatment with the *R*-enantiomer, glutamate rose from 0.67 $\pm$ 0.35 to 2.02 $\pm$ 1.27 μmol/l. The effect of the *R*-enantiomer was not significantly different ($P = 0.19$) from that of vehicle.

3.5. Glutamine

During illumination, extracellular glutamine decreased slightly and transiently in all groups (see Fig. 2a and b).

In the control group 1, glutamine rose 1.4-fold from 23.6 $\pm$ 9.7 μmol/l before illumination to 33.7 $\pm$ 10.6 μmol/l at 5 h after the start of illumination (Fig. 2a). In control group 2, glutamine increased 1.3-fold from 13.2 $\pm$ 9 to 17.6 $\pm$ 14.3 μmol/l (Fig. 2b). The basal levels in the two experimental series differed almost two-fold. This observation seemed to be specific for glutamine since the

other amino acids did not exhibit differences. The possible reason remains unclear. Neither lubeluzole nor the *R*-enantiomer exerted statistically significant effects on glutamine changes.

3.6. Taurine

Extracellular taurine started to rise immediately at the start of illumination, at about the time of the first spreading depression, and reached its maximal level within 20 to 30 min after the start (see Fig. 3a and b). Thus, the rise in taurine preceded that in glutamate.

Results from experiment 1 are shown in Fig. 3a. In control group 1, taurine started at a level of 2.77 ± 0.74 μmol/l prior to illumination. During infarct development, it increased to a maximum and then decreased, reaching a final level of 10.75 ± 3.77 μmol/l at the end of the experiment. The reason for the decrease of taurine remained unclear so far. Post-treatment with lubeluzole immediately stopped any further rise in taurine (basal level: 3.01 ± 0.73 μmol/l; final level: 6.02 ± 4.34 μmol/l). The effect of lubeluzole differed significantly ($P = 0.039$) from that of vehicle.

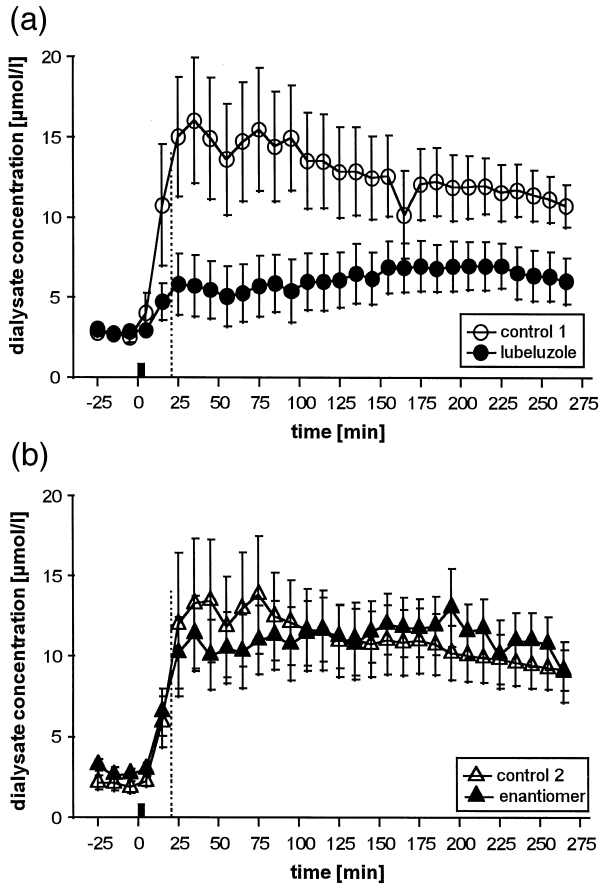

Fig. 3. Dialysate concentrations of taurine before and after photochemical induction of a neocortical infarct in rats. Infarct induction and treatment regimen as in Fig. 1. Data are means ± S.E.M. (a) Effect of lubeluzole (●) versus vehicle (○). (b) Effect of *R*-enantiomer (▲) versus vehicle (△).

Results from experiment 2 are shown in Fig. 3b. In control group 2, taurine rose from 2.18 ± 1.08 to 9.13 ± 3.15 μmol/l. Treatment with the *R*-enantiomer had no effect on the extracellular taurine changes (basal level: 3.27 ± 1.07 μmol/l; final level: 9.01 ± 5.62 μmol/l). Thus, lubeluzole blocked the taurine response, but the *R*-enantiomer left it unaffected.

4. Discussion

Our photochemical procedure induced large thrombotic neocortical infarcts with a clearly detectable core and peri-infarct zone. Although the animals were anesthetized for the entire procedure, the histopathological changes within the infarct or in its surroundings (Fig. 4) were not different from those described in detail for experiments in which the animals had been under halothane anesthesia only for the period of illumination (Van Reempts and Borgers, 1994). It was shown by these authors that, at higher microscopic resolution, the weakly stained peri-infarct area was characterized by viable neurons, surrounded by swollen glial cells. The present results show that the extracellular concentrations of glutamate and taurine showed clear-cut increases inside this peri-infarct zone, whereas glutamine levels did not change. The rise in taurine preceded that in glutamate. Lubeluzole blocked the peri-infarct rise in extracellular glutamate, whereas the *R*-enantiomer produced a small, statistically not significant reduction. Neither compound altered glutamine levels. Lubeluzole completely blocked the taurine response, whereas the *R*-enantiomer left it unchanged.

4.1. Effects of lubeluzole on spreading depressions and extracellular space

Transient DC deflections, including anoxic depolarizations and spreading depressions have been shown to occur in the peri-infarct zone (Nedergaard, 1988; Kocher, 1990; De Ryck et al., 1992; Hansen and Nedergaard, 1993; Nedergaard and Hansen, 1993; Back et al., 1994; Dietrich et al., 1994). Spreading depressions during ischemic conditions are held responsible for the enhancement of ischemic damage in the peri-infarct area (Iijima et al., 1992; Nedergaard and Hansen, 1993; Mies et al., 1994). However, in the photochemical stroke model, the number of spreading depressions in the peri-infarct zone was not affected by lubeluzole (present experiments; see also De Ryck et al., 1995b; Buchkremer-Ratzmann and Witte, 1997). Nevertheless, lubeluzole reduced infarct size, improved the neurological outcome and prevented the impairment of the paired-pulse inhibition in the peri-infarct area of the photochemical stroke model (De Ryck et al., 1996; Buchkremer-Ratzmann and Witte, 1997).

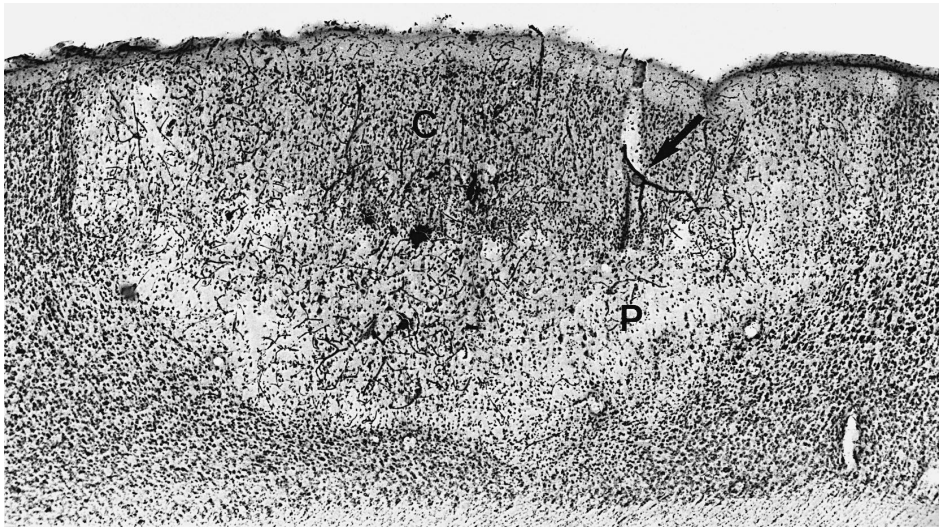

Fig. 4. Azure eosin-stained 100 μm coronal section through a photochemical lesion. The thrombotic core (C), which contains several congested vessels (arrow), can be clearly differentiated from the weakly stained surrounding penumbra (P).

We selected urethane for anesthesia, because urethane does not alter KCl-induced spreading depressions (Bures et al., 1974; Lehmenkühler, 1990; Scheller et al., 1992; Herreras and Somjen, 1993). Since urethane does not affect mechanisms of generation or propagation of KCl-induced spreading depressions, it is conceivable that it did not interfere with spreading depressions induced by the photochemical infarct.

During spreading depression, the extracellular space has been shown to shrink by half (Hansen, 1985; Lehmenkühler, 1990; Scheller and Kolb, 1991; Nicholson, 1993). However, several findings argue against the view that the increases in extracellular glutamate and taurine observed in the present experiments are due to spreading depression-related shrinkage of extracellular space in the peri-infarct zone. In our study, peri-infarct glutamate continued to rise and taurine levels remained high during the last 3 h of the experiment, when spreading depressions no longer occurred and the extracellular potential had returned to normal. Furthermore, if a two-fold reduction of the extracellular space had occurred in our experiments, we should have observed a parallel increase in extracellular glutamate and taurine concentrations. Instead, extracellular glutamate and taurine levels showed different profiles of time-dependent increases. Since lubeluzole blocked the taurine response and limited the rise in glutamate without changing the time course of spreading depressions, it is also most unlikely that the compound exerted its effects by directly restoring the extracellular space.

4.2. Time-course of peri-infarct responses

The rise in glutamate started between 20 to 30 min after infarct induction and continued during the whole experimental period. In contrast, the onset of the taurine response was fast, coinciding with the appearance of the first spreading depressions during infarct induction. These results suggest that the extracellular increase in glutamate might be a delayed event, as has been observed by others (Obrenovitch, 1996; Taguchi et al., 1996). A similar sequence of events, i.e., rise of taurine preceding the increase of glutamate, was observed during potassium-induced cell swelling (Kimelberg et al., 1995) and during complete ischemia (Scheller et al., 1989). In the latter case, taurine started to rise in the extracellular space immediately at the onset of ischemia, whereas glutamate rose after the anoxic DC shift had occurred — i.e., when the cellular energy stores are nearly exhausted (Scheller et al., 1989).

4.3. Clinical relevance of the increase of glutamate

Increases in extracellular concentration of glutamate are related to the degree of cerebral ischemia. In the penumbral zone of an infarct induced by middle cerebral artery occlusion in cats, glutamate remained at near normal or only slightly elevated levels as long as the blood flow did not decrease below the ischemic threshold (Matsumoto et al., 1993). However, persisting ischemia caused large increases in glutamate and irreversible damage. Thus, glutamate levels in the peri-infarct zone can be expected to be lower than those in the infarct core.

Extracellular glutamate levels have been measured in patients undergoing tumor resection (Hillered et al., 1990). Prior to resection, the dialysate levels of glutamate were about 2 μM. After resection, when the tissue was ischemic, the glutamate levels rose 2 to 10-fold. Similar observations have been reported by Bullock et al. (1995). Increases in amino acids have been observed after increases in intracranial pressure, and after flow disturbances, vasospasm, coma or brain death. The conditions for these measurements, microdialysis probes of 2–5 mm length, 20–30 kDa cut-off and a flow rate of 2 μl/min,

were adequately comparable to those of the present investigations (Goodman et al., 1995; Hamberger et al., 1995; Kanthan and Shuaib, 1995; Kawamata et al., 1995). Dialysate glutamate levels correlated with neurological outcome. Patients with glutamate levels around or below 6 μM had a good outcome; those with moderate disabilities had levels from 6.5 to 7.5 μM; and those with poor outcomes had levels above 20 μM (Hillered and Persson, 1995). Thus, the presently observed glutamate levels in the peri-infarct zone were not as high as those in ischemic or irreversibly damaged tissue. It is conceivable that the combination of a small peri-infarct zone with a steep gradient from dead to normal tissue (Van Reempts and Borgers, 1994) and a relatively large microdialysis probe may have underestimated the extracellular concentrations of glutamate immediately adjacent to the infarct edge. Nevertheless, the glutamate levels we now measured in the peri-infarct zone were in the range observed in moderately disabled patients.

4.4. Effects of lubeluzole on glutamate

Lubeluzole blocked the peri-infarct glutamate response, reducing extracellular concentrations of glutamate in the peri-infarct zone from moderately elevated to near normal levels. In accordance with predictions based on the above-mentioned clinical observations, lubeluzole-induced reduction of extracellular glutamate levels is associated with improved neurological outcome in the same photochemical stroke model (De Ryck et al., 1996).

Intravenous post-treatment with lubeluzole suppressed the rise in glutamate in a stereospecific manner. This result is in line with other in vivo findings. For instance, intravenous post-treatment with lubeluzole stereospecifically rescues sensorimotor function and reduces infarct size after photochemically induced infarcts in the motor cortex of rats (De Ryck et al., 1995a, 1996). Likewise, lubeluzole, but not the *R*-enantiomer, restores GABA-mediated paired-pulse inhibition, which is abolished inside a 6-mm wide peri-infarct zone surrounding a photochemically induced neocortical infarct in rats (Domann et al., 1993; Buchkremer-Ratzmann and Witte, 1997).

It could be asked whether this effect is due to presynaptic inhibition of glutamate release or to enhanced reuptake of glutamate. Results from whole-cell voltage clamping have demonstrated that lubeluzole blocks the Na^+ current within the micromolar range (Osikowska-Evers et al., 1995). However, lubeluzole-induced Na^+ channel blockade is not stereospecific (Ashton et al., 1997) and, therefore, not sufficient to explain lubeluzole's stereospecific blockade of the peri-infarct glutamate response. Further, lubeluzole does not appear to affect the reuptake of dopamine, norepinephrine, serotonin and arginine (Lesage et al., 1996). This could mean that the lubeluzole-induced, stereospecific blockade of peri-infarct glutamate is based on a stereospecific neuroprotective mechanism not necessarily related to release or reuptake processes. It is probably also not related to interference of lubeluzole with glutamine metabolism, since glutamine levels remained unchanged. On the other hand, it has been demonstrated that lubeluzole stereospecifically down-regulates the glutamate-activated, nitricoxide synthase pathway (Lesage et al., 1995, 1996) and stereospecifically blocks the neurotoxicity induced by nitric oxide donors (Maiese et al., 1997). In this regard, it has been shown that NO synthase pathway modulation may affect neurotransmitter release (Montague et al., 1994; Nei et al., 1996), which may be in accordance with the present results. Thus, the effect of lubeluzole on glutamate release may occur indirectly, via NO-mediated pathways.

4.5. Effects of lubeluzole on taurine

Taurine, vesicularly released, may function as a putative inhibitory transmitter in hyper-excitatory states (Wheler et al., 1979; Pasantes-Morales et al., 1982; Waniewski et al., 1994). As lubeluzole normalizes neuronal excitability in the peri-infarct zone as shown by the restoration of the paired-pulse inhibition (Buchkremer-Ratzmann and Witte, 1997), it is unlikely that blockade of the taurine response leads to diminished inhibition in the peri-infarct zone. Consequently, it is conceivable that lubeluzole's modulation of the taurine response affects another mechanism of taurine release. Taurine is nonvesicularly released in response to osmotic imbalances (Pasantes-Morales and Schousboe, 1988, 1989; Kimelberg et al., 1990; Pasantes-Morales et al., 1994). Cell swelling has been described in the zone surrounding the core of an infarct (Van Reempts and Borgers, 1994). Thus, osmoregulatory mechanisms could be activated in the affected area.

Post-treatment with lubeluzole, but not with the *R*-enantiomer, prevented the extracellular rise of taurine. This suggests that lubeluzole reduces osmoregulatory stress in the peri-infarct tissue. The mechanism of action is still unknown. However, as cellular swelling may constitute an important stage in the cascade leading to cell death, prevention of excessive energy expenditure, normally required for volume regulation, may contribute to neuroprotection. In this regard, it is interesting to note that lubeluzole reduces glial swelling in hippocampal slices made edematous by veratridine (Ashton et al., 1997). The latter effect might be attributed to the Na^+ channel blocking properties of lubeluzole. However, as mentioned above, lubeluzole blocks Na^+ channels in a non-stereospecific way in vitro (Osikowska-Evers et al., 1995; Ashton et al., 1997) in contrast to its blockade of the taurine response, which is stereospecific. Whether or not the latter effect is related to its stereospecific modulation of the NO synthase pathway is unknown.

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