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# Influence of nitric oxide synthase activity on amino acid concentration in the quinolinate lesioned rat striatum: A microdialysis and histochemical study

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**Summary.** The influence of nitric oxide synthase (NOS) activity on the KCl-evoked amino acid concentrations was investigated by *in vivo* microdialysis in the striatum in a rat model of excitotoxic lesion. Basal microdialysate levels of amino acids decreased during the quinolinic acid-induced neurodegeneration process, except for glutamine that increased initially and returned to control values 30 days after quinolinic acid exposure. KCl-evoked increase of extracellular amino acid concentration was reduced due to NOS activity in the striatum of both controls and lesioned animals, except for 120 days after quinolinic acid injection. These changes of amino acid concentrations in microdialysates correlated with the known biochemistry of the consecutive domineered cell types during the lesion process as revealed by histochemistry for NOS, NADPH-diaphorase, GFAP and isolectin B4. The present data provide direct evidence that NOS activity can modulate extracellular amino acid concentrations in the striatum not only under physiological conditions, but also during a pharmacologically induced lesion process and, thus, suggests that nitric oxide affects neurodegeneration via this pathway.

**Keywords:** Amino acids – Brain microdialysis – HPLC – Immunohistochemistry – Arginine – Glutamate – Neurotoxicity

## Introduction

Nitric oxide (NO), a gaseous radical biomolecule, is a diffusible intercellular signaling substance (Bredt and Snyder, 1992), possibly a kind of spatial second messenger, which coordinates paracrine activity among groups of neurons and glia (Schulman, 1997). Nitric oxide is synthetized from L-arginine in an NADPH-dependent reaction by NO synthase (NOS, EC 1.14.13.39), an enzyme known to occur in different isoforms in the brain. The inducible NOS isoform (iNOS) is typical for glial elements (Murphy et al., 1995; Park and

Murphy, 1994; Simmons and Murphy, 1992), whereas the neuronal (nNOS) and endothelial enzymes were originally identified as constitutive in neurons (Bredt et al., 1991) and vascular endothelium (Lamas et al., 1992), respectively. For instance in the striatum the nNOS isoform is predominantly located in medium aspiny interneurons (Kharazia et al., 1994), a small subpopulation of neurons that also express N-methyl-D-aspartate receptors (East et al., 1996). The main target of NO is proposed to be the soluble guanylyl cyclase (EC 4.6.1.2; Henry et al., 1993) that produces cyclic GMP (East and Garthwaite, 1991; Fedele et al., 1996).

It has been reported that NO plays an important role in several pathological processes, including neuronal death induced by focal cerebral ischemia (Nowicki et al., 1991; Huang et al., 1994), NMDA receptor-mediated neurotoxicity (Garthwaite et al., 1989; Dawson et al., 1995; Dawson et al., 1993b; Strijbos et al., 1996), the mediation of cytotoxic effects in inflammatory diseases (Milstien et al., 1994), and memory impairment during aging (Yamada et al., 1996). Furthermore, NOS induction potentiates excitotoxic injury (Hewett et al., 1994; Choi, 1994; Dawson et al., 1993a). Thus, the inhibition of NOS may protect against neurotoxicity induced by excitotoxicity or energy depletion (Schmidt et al., 1995; MacKenzie et al., 1995; Calka et al., 1996; Schulz et al., 1996; Matthews et al., 1997; Dawson et al., 1993a).

Physiological and pathophysiological mechanisms involving NO in the nervous system are proposed to be accompanied by the modulation of extracellular amino acid concentrations (Guevara-Guzman et al., 1994; Kendrick et al., 1996; Meffert et al., 1996). NOS activity and cyclic GMP content in brain tissue can modulate basal and potassium stimulated amino acid concentrations in the extracellular fluid (Guevara-Guzman et al., 1994; Segieth et al., 1995), but the discrimination between different cellular sources (e.g. neurons or glia cells?) of any of the extracellular amino acid concentrations pools (transmitter, metabolic or osmotic) remains to be clarified.

In the present study acute *in vivo* microdialysis was used to investigate the influence of NO on the KCl-evoked extracellular concentrations of different amino acids during an excitotoxically induced lesion process in the rat striatum. The lesion was induced by local exposure to the NMDA-receptor agonist quinolinic acid (QUIN) which is a widely used (Strijbos et al., 1996; Moroni, 1999), histologically well characterized degeneration model (Beal et al., 1991; Schmidt et al., 1995; Calka et al., 1996). Here we discuss the microdialysis results in the context to histological alterations of the different cell populations within the QUIN lesioned tissue. Additionally, we investigate whether the NO/cyclic GMP-pathway is affected by experiments utilizing 8-bromo-cyclic GMP.

## Material and methods

HPLC solutions were prepared with water for HPLC (J. T. Baker B. V., Deventer, The Netherlands). All chemicals used were of analytical grade, and the solvents methanol and acetonitrile (J. T. Baker B. V., Deventer, The Netherlands) of chromatographic grade.

Ortho-phthalaldehyde was purchased from Carl Roth GmbH & Co. (Karlsruhe, Germany), 8-Br-cGMP from Biomol (Hamburg, Germany) and aspartate, glutamate, glutamine, arginine, citrulline, norleucine, L-NAME, D-NAME and 3-mercaptopropionic acid from Sigma (St. Louis, MO, USA).

Male Wistar rats (Tierzucht Schönwalde, Germany) weighing 220–350 g were housed under a standard diurnal schedule with a 12 h light/12 h dark cycle and free access to water and food. All animal experiments were approved by the Government Committee on Animal Care of the state of Sachsen-Anhalt. Experiments were conducted during the light period of the light/dark cycle. Animals were anasthetized with chloral hydrate (525 mg/kg, i.p.), placed in a Kopf stereotaxic frame, and the skull was exposed. The striatum was lesioned by stereotaxic injection of 1.5 $\mu$l of a 10 mg/ml QUIN-solution (90 $\mu$moles, coordinates: AP 1.0 mm, ML 2.6 mm relative to bregma, 4.8 mm below the dura (Paxinos and Watson, 1986) at a flow rate of 1 $\mu$l/min using a microfusion pump (CMA/100, Carnegie Medicine, Stockholm, Sweden). Either immediately after the QUIN-lesion procedure or 2 days before microdialysis, an intracerebral guide cannula was positioned above the striatum (from bregma: AP 1.0 mm, ML 2.6 mm, and 2.3 mm below the dura) and secured to the skull with anchor screws and dental cement. Rats were allowed to recover in individual home cages for at least 2 days. 30 min before the microdialysis experiment the rats were placed in a special cage that allowed the collection of microdialysis samples in awake and freely moving animals. Each microdialysis probe (CMA/11, 3 mm, Carnegie Medicine, Stockholm, Sweden) was tested for *in vitro* recovery in control perfusion medium (120 mM NaCl, 5 mM KCl, 1.2 mM $MgCl_2$, 1.2 mM $CaCl_2$ in a 15 mM sodium phosphate buffer, pH 7.4) containing amino acid standard concentrations, before being inserted through the guide cannula into the striatum. Control perfusion medium or medium for KCl-stimulation (5 mM NaCl, 120 mM KCl, 1.2 mM $MgCl_2$, 1.2 mM $CaCl_2$ in a 15 mM sodium phosphate buffer, pH 7.4) were prepared freshly, passed through a 0.2 $\mu$m filter and perfused at a flow rate of 1 $\mu$l/min by a microperfusion pump (CMA/100, Carnegie Medicine) with a liquid switch (CMA/111, Carnegie Medicine). After a 4 h equilibration period, consecutive dialysis samples were collected in 10 min intervals. Five baseline samples were taken prior to a 10 min challenge with the high potassium medium, followed by 6 further samples obtained with control perfusion medium. Then a second 10 min KCl-challenge was combined with either the NOS inhibitor $N_G$-nitro-L-arginine methyl ester (L-NAME, 20 mM), the inactive isomer $N_G$-nitro-D-arginine methyl ester (D-NAME, 20 mM), or the cyclic GMP analogue 8-bromoguanosine-3′,5′-cyclic monophosphate sodium salt (8-Br-cGMP, 1.2 mM). This was followed, again, by six sample periods with control perfusion medium. Figure 1 illustrates the experimental design.

Five $\mu$l of the microdialysates were mixed with 2.5 $\mu$l of the internal reference standard (norleucine). An automated precolumn Ortho-phthalaldehyde derivatization procedure was employed as described previously (Böckelmann et al., 1998). Amino acid derivatives were identified by their retention time relative to the reference peak and that of preceding amino acid standards enclosed in the analysis. Quantification was done by measuring peak areas relative to the standards and normalized by the norleucine peak area.

The limitations of the microdialysis technique (Westerink et al., 1987; Justice, 1993) were taken into account by correction of the systematic error of the amino acid levels for the *in vitro* recovery across the dialysis membrane (Menacherry et al., 1992) and expressed as either absolute concentration ($\mu$M), or percentages of the KCl-stimulated value. In case data were found to fit to Gaussian distribution they were submitted to analysis of variance (ANOVA) followed by Tukey-HSD-test. In all other cases data were tested by non-parametric Kruskal-Wallis ANOVA followed by Mann-Whitney U-test, if appropriate. A $p < 0.05$ was considered to be statistically significant.

At the end of the microdialysis experiments both the location of the dialysis probe and the QUIN-induced lesion were histologically analyzed. Anaesthetized animals were intracardially perfused with a 4% paraformaldehyde, 0.4% glutaraldehyde, and 10% sucrose 0.1 M sodium phosphate buffer (pH 7.4). After postfixation in 0.4%

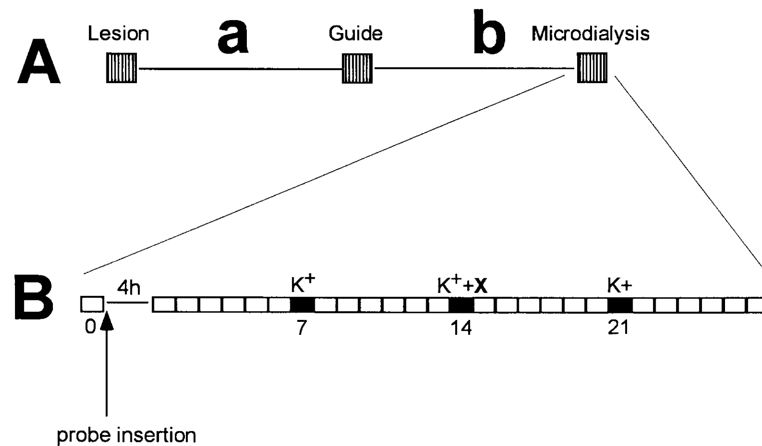


**Fig. 1.** Experimental protocol for treatment of the animals and collecting of the microdialysis samples. **A** Intervals between lesion and guide cannula implantation (**a**) were 0 days (groups 2 and 7 days), 27 days (group 30 days) and 117 days (group 120 days) until microdialysis. The interval between guide cannula implantation and microdialysis (**b**) was always one day except for the group 7 days (5 days). **B** Collection of 10-min microdialysis samples (white and black squares). Sample 0 was collected to estimate the in vitro recovery. Except for the collection of samples 7, 14 and 21 the dialysis medium consisted of an isotonic medium (white squares). During the collection of the latter three samples (black squares) the dialysis medium comprised either 120 mM KCl alone (samples 7 and 21) or 120 mM KCl and (*X*) either L-NAME or D-NAME or 8-Br-cGMP (sample 14)

paraformaldehyde +30% sucrose, the brain was cut in a cryostat into 20 $\mu$m thick sections and stained with cresyl violet. To identify single cell types (Schmidt et al., 1995) additional sections of the same brains were stained for NADPH-diaphorase activity and nNOS (polyclonal antibody, rabbit, Eurodiagnostica, Malmø, Sweden). GFAP-immunostaining (monoclonal antibody, Boehringer Mannheim, Germany) or isolectin B4 labeling (Sigma, Deisenhofen, Germany) were used to visualize astroglia and microglia/macrophages, respectively.

## Results

The individual amino acid recovery *in vitro* of the microdialysis probes CMA/11 ranged from 7.3 ± 1.2% to 13.9 ± 5.7% of the standard concentration. The delay between the start of dialysis and the collection of stable baseline values of amino acids in control rats was approx. 240 min (Fig. 2). Therefore the start of sampling was fixed at 4 hours after the begin of the brain dialysis. No difference was found between lesioned and control animals when the amino acid levels at different time points across the development of the QUIN-induced lesion were compared to their respective controls (data not shown).

The basal concentrations of aspartate, glutamate, citrulline and arginine decreased during the lesion process when compared to control values ($p < 0.05$, randomized block ANOVA followed by Tukey-HSD test, except for arginine on day 7 and glutamate as well as citrulline on days 7 and 120 after QUIN; Fig. 3). The basal concentrations of these four amino acids reached

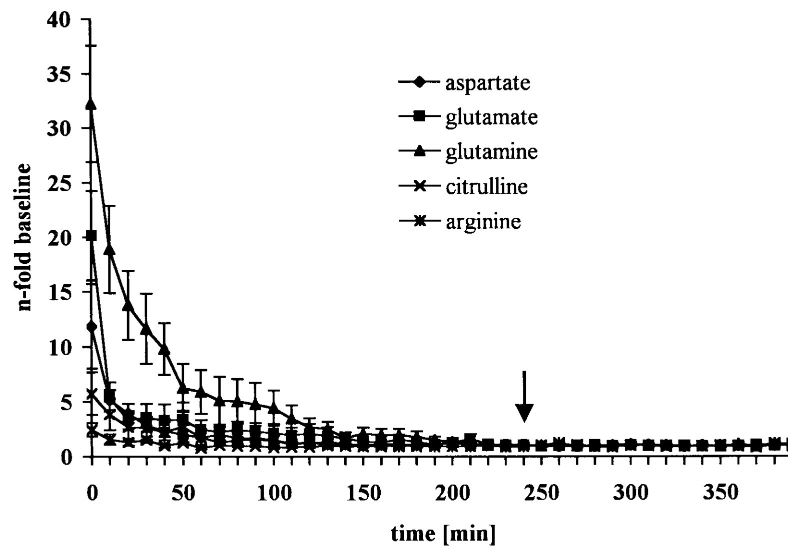


**Fig. 2.** Time course of amino acid concentrations starting after insertion of the microdialysis probe in control animals as compared to baseline concentrations of each amino acid (means ± S.E.M., n = 5). The arrow indicates the time point considered as stable baseline concentration used as time 0 for all further experiments

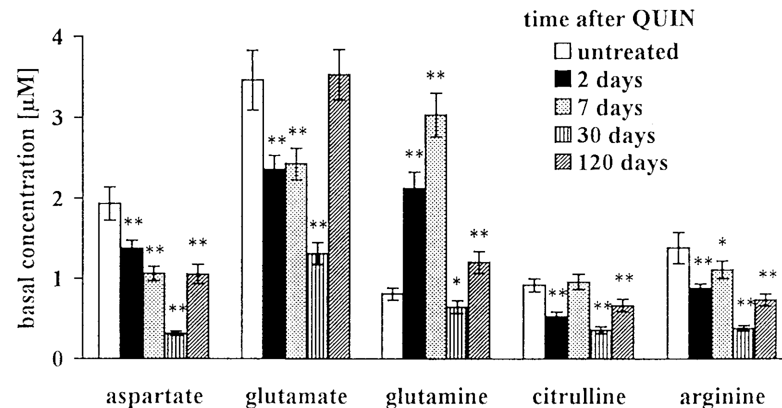


**Fig. 3.** Basal concentrations of amino acids in striatal microdialysates of control rats (n = 17), and on day 2 (n = 15), 7 (n = 17), 30 (n = 15), and 120 (n = 15) after exposure to QUIN, corrected for the amino acid recovery by the microdialysis probes as determined *in vitro*. Data are presented as means ± S.E.M. *$p < 0.05$, **$p < 0.01$ (randomized block ANOVA followed by Tukey-HSD test)

their minimum on day 30 after intoxication and increased again 120 days after QUIN-injection (Fig. 3). Whereas glutamate levels returned to initial control values, levels for aspartate, citrulline and arginine did not fully recover.

In contrast glutamine values increased during the first week of QUIN-intoxication and returned to control values 30 days after intoxication (Fig. 3).

Basal concentrations of both amino acids tended to recover 120 days later (n = 15), but remained at lower levels than in control rats. Perfusion with high

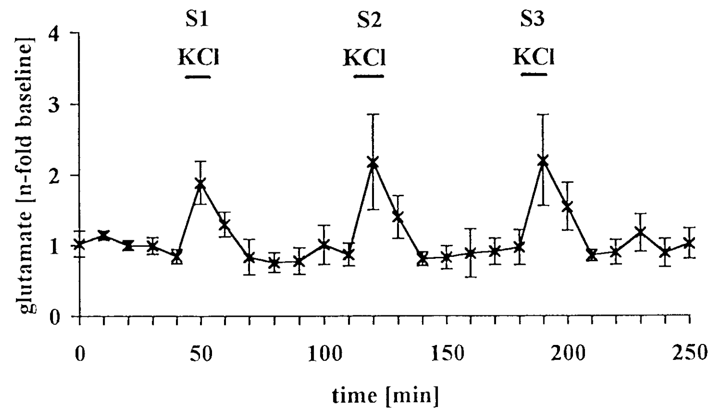


**Fig. 4.** Time course of glutamate concentrations (means ± S.E.M.) during microdialysis in control rats (n = 3). Samples were taken in 10 min intervals. Bars above the graph indicate perfusion with high KCl containing medium (*S1–S3*) via the microdialysis probe

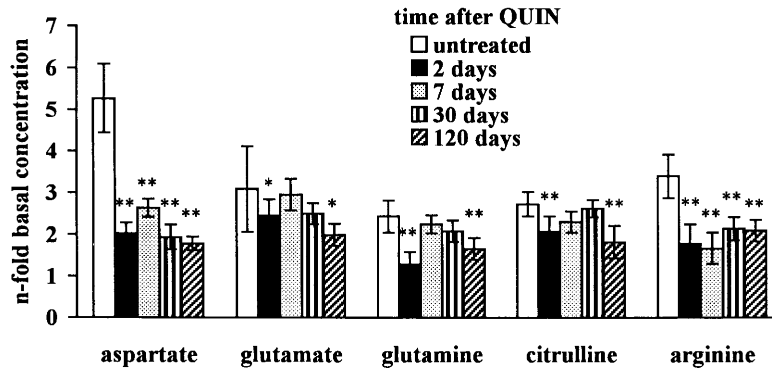


**Fig. 5.** KCl-evoked concentrations (at S1) during the lesion process (n = 12 to 14 each group). Data are presented as n-fold basal concentrations (means ± S.E.M.). $^{*}p < 0.05$, $^{**}p < 0.01$ (Kruskal-Wallis nonparametric ANOVA followed by Mann-Whitney U-test)

KCl resulted in significantly increased amino acid concentrations in the dialysates of all experimental groups (for example glutamate concentration in control rats, Fig. 4).

Amino acid levels during exposure to high KCl were increased in control rats by the following factors: aspartate 5.27 ± 0.83, glutamate 3.09 ± 1.03, glutamine 2.43 ± 0.38, arginine 3.40 ± 0.52, and citrulline 2.73 ± 0.29. The extend of which KCl affected amino acid concentrations was lower in QUIN-lesioned animals than in controls at all measured time points, except for glutamate, glutamine and citrulline on day 7 and after 30 days (Fig. 5).

KCl-evoked aspartate concentration was decreased in the presence of L-NAME in control rats and on day 120 after QUIN-induced lesions (Table 1). On day 2 after QUIN-intoxication the KCl-induced concentration of both, glutamate and aspartate, were not affected by L-NAME. The KCl-evoked

**Table 1.** Influence of the NO/cGMP-pathway on the KCl-evoked concentrations of amino acids (mean of the 2nd $K^+$-stimulation with the drug/mean of the 2nd $K^+$-stimulation alone). Asterisks indicate significant differences between $K^+$-stimulation alone and treated with L-NAME, D-NAME or 8-Br-cGMP ($n = 3 - 4$, Mann-Whitney U-Test, * $p < 0.05$)

| Amino acid | Day | $K^+$ + L-NAME | $K^+$ + D-NAME | $K^+$ + 8-Br-cGMP |
|---|---|---|---|---|
| aspartate | control | 0.48* | 0.95 | 0.84 |
| | 2 | 1.16 | 1.18 | 0.94 |
| | 7 | 0.77 | 0.68 | 1.07 |
| | 30 | 0.84 | 1.15 | 1.34 |
| | 120 | 0.76* | 1.30 | 1.85 |
| glutamate | control | 0.72 | 1.38 | 0.91 |
| | 2 | 0.55 | 0.92 | 0.53* |
| | 7 | 0.55* | 0.55 | 0.71 |
| | 30 | 2.01 | 1.11 | 1.21 |
| | 120 | 0.66* | 1.26 | 1.29 |
| glutamine | control | 0.92 | 0.93 | 0.67 |
| | 2 | 0.90 | 0.69 | 0.50* |
| | 7 | 0.66 | 0.93 | 1.00 |
| | 30 | 0.85 | 0.95 | 1.58 |
| | 120 | 0.84 | 1.41 | 0.66 |
| citrulline | control | 0.48 | 1.41 | 1.38 |
| | 2 | 0.44* | 0.77 | 0.93 |
| | 7 | 0.48* | 0.96 | 0.77 |
| | 30 | 0.76 | 0.76 | 2.61* |
| | 120 | 1.08 | 1.48 | 1.57 |
| arginine | control | 0.79 | 1.02 | 0.66 |
| | 2 | 2.33* | 0.65 | 0.62 |
| | 7 | 1.40* | 0.67 | 0.86 |
| | 30 | 2.14* | 1.15 | 1.63 |
| | 120 | 1.00 | 0.67 | 0.69 |

concentration of glutamate was reduced by L-NAME on day 7 and day 120 of QUIN-induced lesions, but this effect was not observed at day 30 after the QUIN-induced lesions. KCl-stimulated glutamine concentration was not influenced by NOS-inhibition in all experimental groups (Table 1).

L-NAME tended to decrease the KCl-evoked citrulline, but not the arginine values in the control rat striatum ($n = 5$, Table 1). L-NAME potentiated the high-KCl-stimulated arginine concentration and decreased that of citrulline 2, 7 and 30 days after intoxication ($n = 3$, 4, and 3, respectively). 120 days after lesioning ($n = 3$), however, L-NAME failed to affect the KCl-induced concentration of both amino acids.

D-NAME was found to have no effect on KCl-evoked amino acid concentrations in any of the experimental groups, but 8-Br-cGMP decreased both the glutamate and glutamine concentrations at day 2 after QUIN-intoxication and increased citrulline at day 30 after QUIN-induced lesion (Table 1).

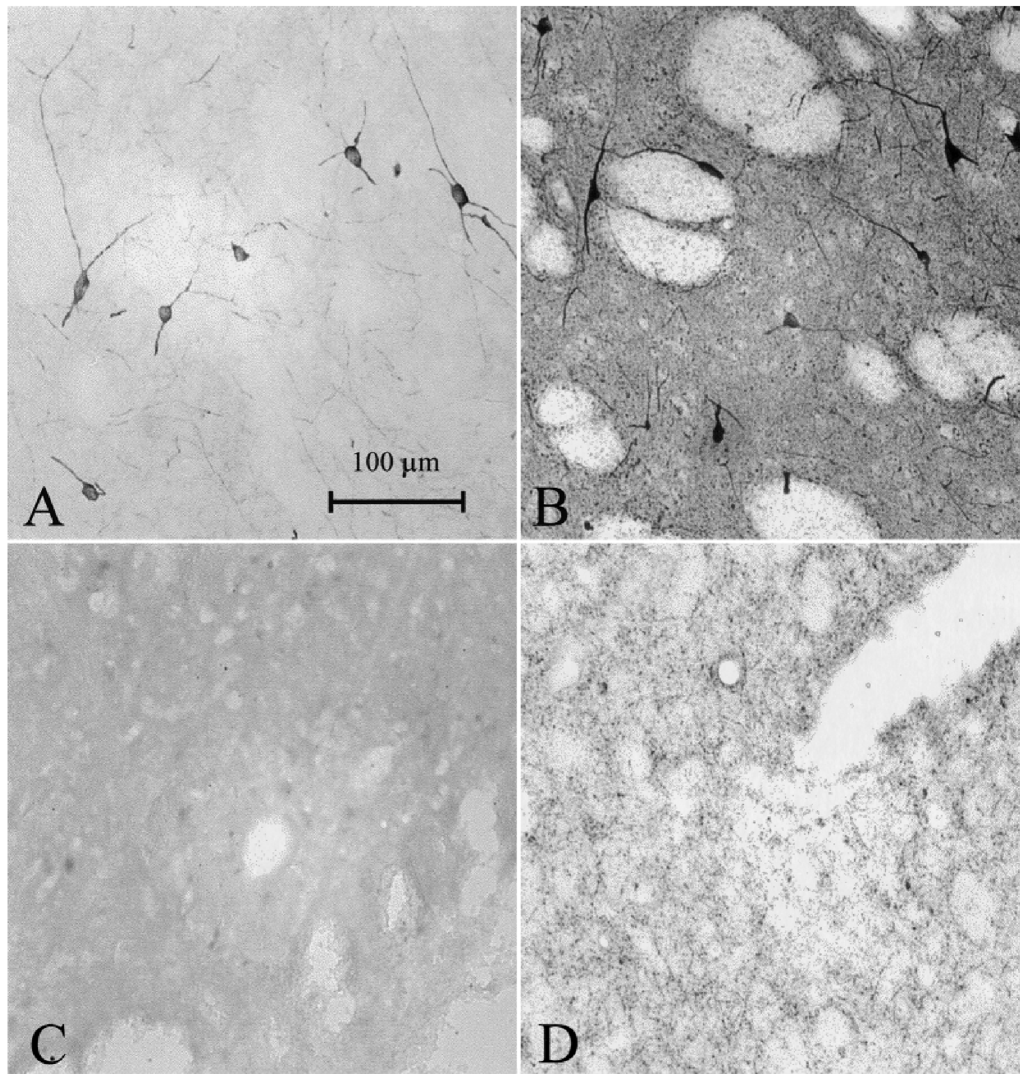


**Fig. 6.** Photomicrographs depicting the striatum near the microdialysis cannula (**A, B**) in control rats, and (**C, D**) 2 days after QUIN-induced neurodegeneration. **A**, **C** nNOS-immunostained neurons; **B**, **D** labeling of neurons by NADPH-diaphorase reaction. The scale bar in **A** is applicable in all subfigures. Note the loss of nNOS- and NADPH diaphorase-positive neurons at day 2 after lesioning

A massive loss of neurons was seen in the striatum 2 days after exposure to QUIN when standard histological staining was used. Similar neuronal loss was found when the sections were stained for nNOS-immunoreactivity (Fig. 6A–D). Parallel to the depletion of neurons a marked intensivation of isolectin-B4 on day 2 (Fig. 7A, B) and GFAP-staining on day 7 (Fig. 7C, D) indicated an activation of glial cells. Activated astroglia and microglia/macrophages revealed a progressive immunostaining for inducible NOS and labeling by NADPH-diaphorase reaction (Fig. 8, A, B), whereas quiescent glia was not stained (data not shown). The striatal area in the sections was

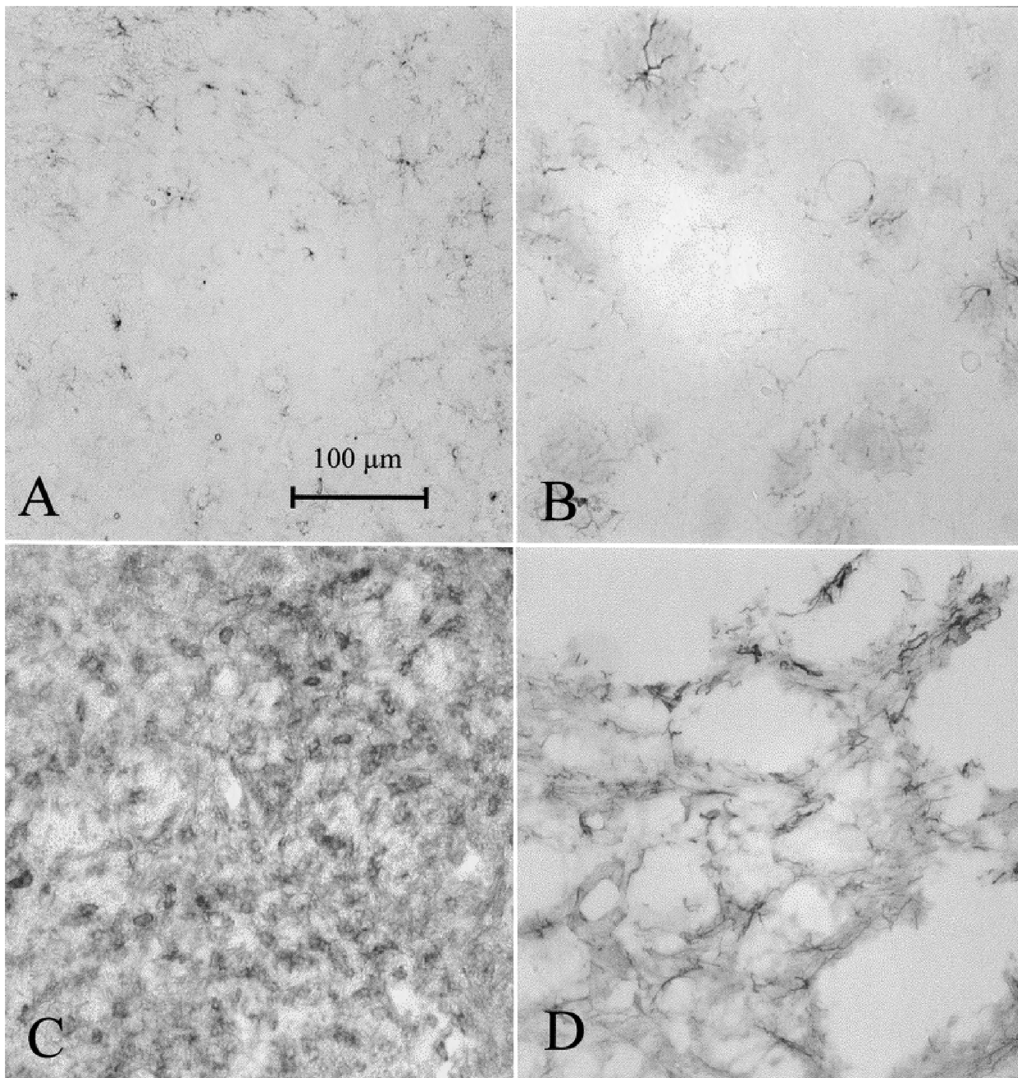


**Fig. 7.** Photomicrographs of the striatum near the microdialysis cannula (**A, B**) in control rats, (**C**) 2 days, and (**D**) 7 days after QUIN-induced striatal lesion. **A**, **C** Isolectin B4-immunostaining of microglial cells; **B**, **D** GFAP-immunostaining of astroglia. The scale bar in **A** is applicable in all subfigures. Note the increased isolectin B4-staining and GFAP-immunoreactivity at 2 and 7 days, respectively, after lesioning

strongly reduced 120 days after QUIN-injection (Fig. 9). Only GFAP-stained glial cells appeared to be substantially less activated, and could no longer be labeled for NADPH-diaphorase activity or NOS-immunoreactivity.

## Discussion

Microdialysis technique has been rarely used in excitotoxically lesioned tissue, so far. There are a few reports on increased amino acid concentrations during

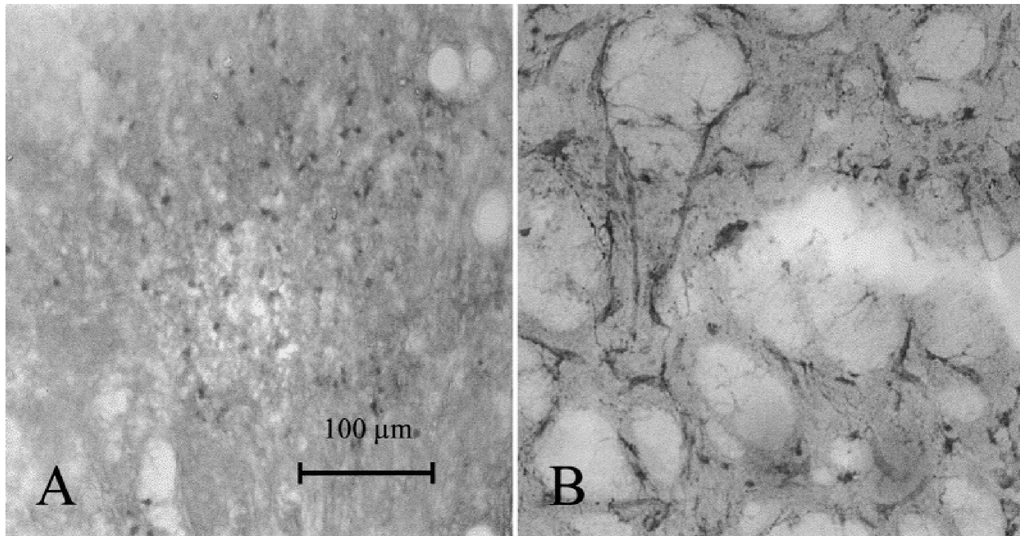


**Fig. 8.** Photomicrographs of the rat striatum near the microdialysis cannula 7 days after QUIN-induced striatal lesion. **A** Immunostaining for inducible NOS of microglial cells. **B** Labeling of blood vessels and glial cells by NADPH-diaphorase reaction. The scale bar in **A** is applicable also in **B**. Note the activation of iNOS-immunoreactive microglia and NADPH diaphorase-positive astroglia and blood vessels

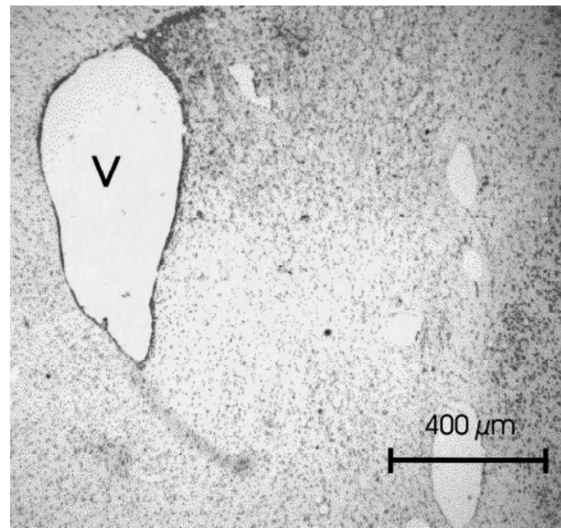


**Fig. 9.** Photomicrograph of the rat striatum near the microdialysis cannula 120 days after QUIN-induced striatal lesion. A cresyl violet-staining of the shrunken striatum (glia scar). *V* ventricle

the first hours following local application of the glutamate receptor agonist QUIN (Bakker and Forster, 1991; Fedele and Foster, 1993). Both basal and KCl-induced amino acid concentrations measured the striatum of control rats in the present study are in good agreement with data of the literature

(Kendrick et al., 1996; Guevara-Guzman et al., 1994). Furthermore consistent with our findings, the KCl-induced concentration of aspartate was reported to be reduced by NOS-inhibition in control rats (Guevara-Guzman et al., 1994; Kendrick et al., 1996). We extended the examination of amino acid concentrations in the lesioned striatum up to 120 days after QUIN injection. This made it possible to compare the histological outcome of the excitotoxically lesion with the influence of NOS-activity on the local, extracellular amino acid concentrations during the QUIN-induced degeneration process and, thus, to investigate the impact of NO on fluctuations of amino acid concentrations in the context to changes of cellular populations during degeneration. Unfortunately, histological analysis of the QUIN-induced tissue damage had to be performed for technical reasons for each treatment group separately. Therefore, we were not able to perform a correlation analysis between the amino acid concentration in the microdialysates and selected histolochemical markers.

Although it is widely accepted that microdialysis is a reliable tool for measuring changes in striatal concentrations of amino acids (Westergren et al., 1995; Obrenovitch et al., 1995) a major problem of the introduction of the microdialysis probe into the brain tissue concerns the acute damage of the blood-brain barrier. To minimize the resulting elevated extracellular amino acids levels (Fig. 2; see also Benveniste, 1991) we delayed the start of the experiments for 4 hours after insertion of the microdialysis probe into the brain tissue. At this time point, stable basal concentrations were achieved in both the control and the lesioned striatum (Fig. 2).

Basal concentrations of amino acids were significantly altered 2 days following local intoxication by QUIN. The observed decrease of selected amino acids occurred in parall to the dramatic neuronal loss as histologically demonstrated in the present study (Fig. 6) and was described in more detail by previous reports (Calka et al., 1996; Schmidt et al., 1995). Based on these histological observations it can be hypothesized that the increased basal glutamine concentration reflects a disturbed glutamine-glutamate cycle (Westergaard et al., 1995; Schousboe et al., 1992) as a consequence of the tissue damage. Furthermore, a progressive accumulation of highly activated microglia/macrophages was histologically identified in the degenerating tissue (Fig. 7; see also Koshinaga and Whittemore, 1995; Calka et al., 1996; Schmidt et al., 1995; Marty et al., 1991) accompanied by an upregulation of iNOS (Calka et al., 1996). Therefore, high iNOS activity might explain why the inhibition of NOS by L-NAME altered substantially the KCl-evoked concentrations of the NOS substrate arginine and its byproduct citrulline (Table 1). Interestingly 2 days after QUIN KCl-induced glutamate and aspartate concentrations were not affected by NOS-inhibition. The reduction of amino acid levels on day 2 is most likely due to the loss of neuronal glutamatergic structures. At this time point the extracellular amino acid concentration in the glial compartments may be relatively low and are not influenced by NO (Schmidlin and Wiesinger, 1995).

Seven days after QUIN-application the lesion contained a decreased number of activated microglia/macrophages and an increased number of

activated astroglial cells, when compared to day 2 (Fig. 7; see also Calka et al., 1996; Dusart et al., 1991; Schmidt et al., 1995). A progressive activation of astroglia resulting in an enhanced basal glutamine concentration and recovery of basal arginine and citrulline values (Fig. 3) can be assumed. The significant effect of L-NAME on the KCl-evoked citrulline and arginine values may be explained by the high NOS activities in the glial and the proliferating endothelial compartments that may conceivably be also the source of the released amino acids.

The lowest basal and KCl-evoked amino acid concentrations were measured 30 days after QUIN-application (Figs. 3 and 5). This is likely to be the result of a decreased number of activated astroglial cells in the center of the lesion. The inhibiting effect of L-NAME on the KCl-evoked citrulline concentration can be explained by a residual NOS activity in glial cells. The low concentrations of basal and KCl-evoked amino acids levels obtained from the lesions may indicate that amino acid transport processes are generally diminished. Furthermore, despite its decreased number, the activated astroglia were the predominant element in the lesioned tissue, and the sustained increase of NOS-staining in astroglia (Schmidt et al., 1995) may explain that inhibition of NOS was still able to diminish KCl-evoked extracellular aspartate concentrations.

Finally, 120 days after QUIN-intoxication, when a glial scar was formed and the striatum was extremely shrunken (Fig. 9; see also Böckelmann et al., 1998) basal amino acid concentrations were higher than 30 days after intoxication but did not return to levels which were found in control animals. The KCl-evoked concentration of all amino acids studied declined compared with levels in control rats apparently due to the total lack of neuronal cell bodies. Although at that time point, a residual NOS-activity in astrocytes (indicated by a weak NADPH-diaphorase staining; Böckelmann et al., 1998) can not be fully excluded, because NOS-inhibition had a moderate, but significant effect on excitatory amino acid concentrations.

The decrease of extracellular excitatory amino acid concentrations by NOS-inhibition during high KCl-exposure was found to be mainly not mediated through a cyclic GMP-coupled mechanism, because cyclic GMP analog 8-Br-cGMP failed to reveal a detectable effect. These results are consistent with our previous report concerning the concentration of taurine (Böckelmann et al., 1998). Moreover, the [$^3$H]aspartate release from rat striatal slice preparations (Reiser et al., 1999) was also shown to be independent from 8-Br-cGMP-exposure. On day 2 after QUIN-intoxication the significant decrease of glutamate and glutamine during high KCl-exposure by 8-Br-cGMP co-exposure is in contrast to their unchanged KCl-evoked concentrations by NOS-inhibition and might be a non-neuronal effect in the lesioned, neuron depleted tissue.

Taken together, our results demonstrate that microdialysis technique is suitable to monitor extracellular amino acid concentration in excitotoxically lesioned tissue. Obviously the fluctuations in the concentration of amino acids parallel the morphological changes during the lesion process. Our results imply that NO is involved in these release patterns of amino acids, both in the

control and in the degenerative striatum. Interestingly, the NO-effect is mainly not mediated by cyclic GMP and basal and the KCl-stimulated amino acid concentrations are largely independent of NOS activity in the glial scar. Further studies are needed to clarify what alternative mechanisms other than the NO/guanylyl cyclase/cyclic GMP pathway are responsible for the NO-effect on extracellular amino acid levels.

## Acknowledgments

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