

# Brain Extracellular Quinolinic Acid in Chronic Experimental Hepatic Encephalopathy as Assessed by In Vivo Microdialysis: Acute Effects of L-Tryptophan

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*Increased brain quinolinic acid (QUIN) levels have been suggested to play a role in hepatic encephalopathy (HE). Previous brain tissue studies have been unable to confirm this hypothesis. Because QUIN is a potent NMDA-receptor agonist, it also is relevant to determine brain extracellular QUIN levels in HE. For this purpose, we assessed frontal neocortical extracellular QUIN levels by in vivo microdialysis in rats subjected to a portacaval shunt (PCS). We also evaluated the acute effects of altered L-tryptophan (L-TRP) availability on brain extracellular QUIN levels.*

*The basal extracellular L-TRP levels were significantly ( $p < .001$ ) higher in the PCS rats than in the sham-operated controls. However, the QUIN level ( $p < .05$ ) and the QUIN to L-TRP ratio ( $p < .01$ ) were significantly lower in the PCS rats. Elevated L-TRP availability increased the QUIN levels to a similar degree in both sham and PCS rats. This study, in conjunction with our previous results, does thereby not support a major involvement of QUIN in the pathogenesis of HE. [Neuropsychopharmacology 15:382-389, 1996]*

**KEY WORDS:** *Hepatic encephalopathy; In Vivo cerebral microdialysis; Kynurenines; Liver disease; Portacaval anastomosis; Quinolinic acid*

The pathogenesis of the neuropsychiatric syndrome associated with a variety of both acute and chronic liver diseases, better known as hepatic encephalopathy (HE), is still unknown. In patients with varying degrees of HE, the cerebrospinal fluid (Ono et al. 1978; Smith et al.

1978; Hutson et al. 1979; Cascino et al. 1982; Rössle et al. 1984), as well as postmortem brain tissue (Jellinger et al. 1978; Bergeron et al. 1989), levels of the aromatic amino acid L-tryptophan (L-TRP) have convincingly been shown to be increased. Elevated L-TRP levels have also been clearly demonstrated in brain tissue (Baldessarini and Fischer 1979; Curzon et al. 1975; Cummings et al. 1976; Mans et al. 1982, 1990; Bengtsson et al. 1991) and in the brain extracellular fluid (Bergqvist et al. 1995b) in portacaval shunted (PCS) rats, a commonly used and non-toxin-induced experimental in vivo model of chronic HE. L-TRP is an essential component in all vertebrate diets, and the pharmacological effects often seen directly as the results of L-TRP augmentation or depletion of the food indicate a central role for cell-to-cell communication promoted by this amino acid. Furthermore, L-TRP is the precursor of many neuroactive compounds such as serotonin, 5-hydroxytryptophol, melatonin, and tryptamine, as well as for compounds metabolized via the kynuren-

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Received August 17, 1995; revised October 31, 1995; accepted November 9, 1995.

ine pathway. Among the kynurenine pathway metabolites, quinolinic acid (QUIN; pyridine-2,3-dicarboxylic acid) seems to be of particular pharmacological and functional importance (for an overview, see Stone 1993). QUIN is an endogenous agonist of the NMDA-receptor, mediating neuronal membrane calcium ion transients and thereby excitatory neurotransmission in the brain (Stone 1993).

In the mid-1980s elevated brain QUIN levels were reported in humans with HE as well as in PCS and acute hyperammonemic rats (Moroni et al. 1986a, 1986b). In a recent study, however, decreased rather than increased plasma and brain tissue QUIN levels were found in PCS rats (Bergqvist et al. 1995a). Furthermore, no parallel change between neurological symptoms in acute hyperammonemia with brain QUIN levels occurred (Bergqvist et al. 1995a). Because QUIN is a potent excitatory receptor ligand in vivo, there is still a possibility that relatively minor elevations of QUIN in the extracellular environment in the brain could play a role in the pathogenesis of HE (Bengtsson 1992; Butterworth 1992).

In the present study, we determined the frontal neocortical extracellular L-TRP and QUIN levels in chronic PCS rats using the in vivo microdialysis technique. First, we determined basal extracellular levels of L-TRP and QUIN 5 to 6 weeks following permanent surgical PCS. Second, we also assessed the acute effects of systemic pharmacological manipulation of L-TRP availability on the extracellular levels of QUIN at this point.

## MATERIALS AND METHODS

### Animals and Surgery

Male Sprague-Dawley rats weighing 175 to 200 g (Møllegaard Breeding & Research Centre A/S, Skensved, Denmark) were used. The animals had free access to tap water and standard laboratory pelleted chow containing 21% crude protein (product R3; Ewos AB, Södertälje, Sweden). No pair feeding of controls was conducted. The rats were housed in groups of three animals in standard macrolone cages with sawdust bedding under climate-controlled conditions for temperature and humidity. The rats were kept in a 12-hour light/dark cycle synchronous with daylight (lights on at 6.00 A.M.). The study was approved by the Animal Ethics Committee at Lund University.

A permanent surgical end-to-side PCS was created with some modifications (Bengtsson et al. 1991; Bergqvist et al. 1995a). The control rats underwent sham operation (sham) identical to the PCS procedure except for the performance of the portacaval anastomosis. The PCS and the sham rats were allowed to recuperate for 5 to 6 weeks after intraabdominal surgery before the microdialysis experiments were performed. The rats were

controlled for body weight at time of the PCS-procedure, thereafter weekly and at termination of the experiments. At time of sacrifice the liver weight was recorded, and the liver-to-body weight ratio was calculated.

### In Vivo Microdialysis

The microdialysis probes were prepared as described by Kalén et al. (1988), using GFE Plus16 (Gambro, Lund, Sweden) hemophane tubing (OD 0.22 mm and ID 0.20 mm) with a molecular cutoff of 7 kDa. The probes were implanted into the frontal neocortex (coordinates relative to bregma: rostral, +3.2 mm; lateral, -1.5 mm; ventral, -2.5 mm; Paxinos and Watson 1982) under halothane anaesthesia (ISC Chemicals Ltd., Avonmouth, England) the day prior to the dialysis procedure. The subsequent day, the probe inlet was attached to a liquid swivel that was connected to a microinfusion system (model CMA/100; Bioanalytical Systems Inc. [BAS], West Lafayette, IN). All microdialysis experiments were conducted on conscious and freely moving animals. The probes were perfused at 2.5  $\mu$ L/minute with an isotonic Ringer's buffer (Pharmacia AB, Uppsala, Sweden; NaCl 147 mmol/L, KCl 4 mmol/L, CaCl<sub>2</sub> 2.4 mmol/L, pH 6.0). No samples were collected during an initial stabilization period of 45 minutes. Then, samples were collected in 15-minute fractions to obtain an adequate baseline. The samples were immediately frozen in liquid nitrogen without addition of any antioxidants and stored at -70°C until the subsequent neurochemical analyses were performed. Not all dialysis samples collected were analyzed for QUIN or L-TRP, but they were used for other kinds of chemical analyses and included in other investigations.

After collection of the baseline fractions, the L-TRP availability was manipulated pharmacologically by systemic administration of a single loading dose of L-TRP (280 mg/kg I.P.; Sigma Chemical Co., St. Louis, MO) dissolved in isotonic saline. The first L-TRP injection was repeated 3 hours later by four injections of lower doses of L-TRP (50 mg/kg each) during the four consecutive hours to follow. The total volume of fluid administered this way during the 6.5-hours experiment was about 2 mL/100 g of body weight. In a number of animals from each group (sham and PCS) L-TRP was replaced by an equimolar amount of saline (Sigma) to account for variables such as possible nonspecific peritoneal irritation and osmotic effects. Thus, four experimental groups denoted as sham-saline ( $n = 5$ ), sham-L-TRP ( $n = 4$ ), PCS-saline ( $n = 4$ ), and PCS-L-TRP ( $n = 4$ ) were investigated.

Diurnal fluctuations in the concentration of brain large neutral amino acids (e.g., L-TRP) are known to exist (Crandall and Fernstrom 1983). Therefore, we also

assessed the basal levels of L-TRP 24 hours after initiating the microdialysis procedure.

### Neurochemistry

**L-Tryptophan.** The dialysates content of L-TRP was determined by a method modified from Hjorth and Sharp (1993) applying a high-performance liquid chromatography (HPLC) assay with electrochemical detection. In brief, the samples were separated at 30°C on a 2.0-mm × 15-cm stainless steel column packed with Ultrasphere ODS 5-μm material (Beckman Instruments Inc., Fullerton, CA). The mobile phase consisted of 0.126 M NaH<sub>2</sub>PO<sub>4</sub> (pH 4.0) containing 0.85 mM EDTA, 0.03 mM sodium n-octyl sulfate, and 10% (vol/vol) HPLC-grade methanol. An aliquot of 20 μL of dialysate was manually injected with a Rheodyne injector (model 7125; Rheodyne Inc., Cotati, CA). The mobile phase was delivered at 0.25 mL/minute through a twin-reciprocating pump (LKB-2150; LKB-Produkter AB, Bromma, Sweden). The L-TRP was detected by a glassy carbon working electrode set at +0.80 V relative to an Ag/AgCl reference electrode (LC-4B; BAS), and the detection signals were recorded on an integrator (SP 4290; Spectra-Physics, San Jose, CA). The detection limit (signal-to-noise ratio, 3:1) for L-TRP was 0.1 pmol.

**Quinolinic acid.** The QUIN content of the dialysate samples was determined according to a method previously described in great detail (Heyes and Markey 1988a, 1988b) using [<sup>18</sup>O]QUIN as internal standard (see also Bergqvist et al. 1995a). In short, after derivatization with hexafluoroisopropanol (HFIP) the samples were analyzed with a gas chromatograph = mass spectrometer (GC = MS; Hewlett-Packard 5988A quadrupole mass filter) operating in an electron-capture negative chemical ionization mode using volatile methane and helium as reagent and carrier gases, respectively. The QUIN-HFIP and [<sup>18</sup>O]QUIN-HFIP derivatives were quantified by peak areas of the molecular anions [mass charge ratio (m/z) of 467 and 471, respectively]. The detection limit of this method (signal-to-noise ratio, 25:1) for QUIN was 0.3 fmol.

### Statistics

All values are expressed as means ± SEM. A probability of less than 5% ( $p < .05$ ) was preset to signify significant differences between groups. All statistical analyses were performed by using the computer software StatView 4.0 (Abacus Concepts, Inc., Berkeley, CA). When two groups were compared, a two-tailed Student's *t*-test was used. When more than two groups were compared, a one-factor analysis of variance (ANOVA) was used. If significance was reached with the ANOVA, a post hoc analysis (Fis-

cher's PLSD-test) was applied to determine which groups differed.

## RESULTS

### Weight Measurements

The administration of L-TRP was shown not to affect the body and liver weight parameters in either the PCS or sham groups. Hence, the data from these measurements were pooled into two groups representing only sham or PCS. At termination of the experiment 5 to 6 weeks postsurgery, there was a significant ( $p < .001$ ) difference in body weight between sham ( $399 \pm 8$  g,  $n = 9$ ) and PCS rats ( $273 \pm 16$  g,  $n = 8$ ). The body weight gain from the time of operation to sacrifice in sham ( $133 \pm 7$  g) and PCS ( $11 \pm 8$  g) indicated that both groups had reached a postsurgical anabolic state by the time the dialysis experiments were performed. A significant ( $p < .001$ ) difference in the liver-to-body weight ratio between the sham rats ( $3.6 \pm 0.1\%$ ,  $n = 9$ ) and the chronic PCS rats ( $2.3 \pm 0.1\%$ ,  $n = 8$ ) was present at sacrifice.

### Basal Frontal Neocortical Extracellular L-TRP and QUIN Concentrations

Five to six weeks following the PCS procedure the extracellular L-TRP levels in the PCS rats were significantly ( $p < .001$ ) higher than in the sham-operated controls (Table 1). The brain extracellular QUIN levels as well as QUIN-to-L-TRP ratios were, however, significantly lower ( $p < .05$  and  $p < .01$ , respectively) in the PCS rats than in the sham rats.

### Effects of an Exogenous Systemic L-TRP Challenge on the Frontal Neocortical Extracellular L-TRP and QUIN Levels

The PCS and sham rats exposed to saline injections displayed a continuous and comparable longitudinal lowering of the extracellular L-TRP during the 6.5-hour course of dialysis (circles in Figure 1). This tendency to-

**Table 1.** Basal Extracellular L-TRP and QUIN Levels (nmol/L) and QUIN to L-TRP ratio (%) in PCS and Sham-Operated Rats 5 to 6 Weeks Following the PCS Procedure

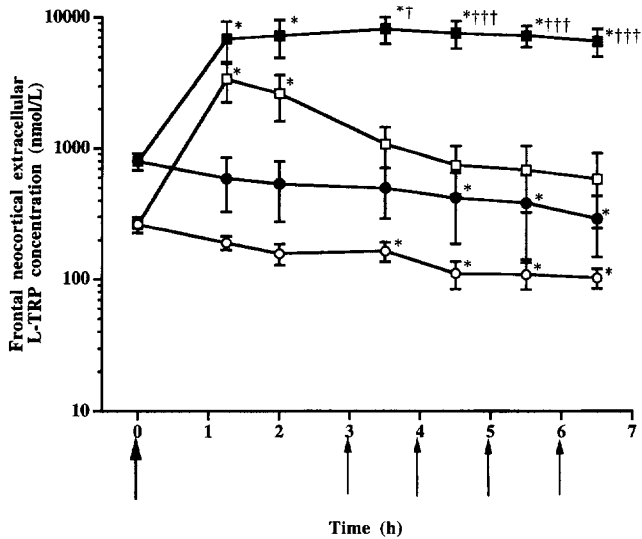
|            | Sham ( $n = 9$ ) | PCS ( $n = 8$ ) |
|------------|------------------|-----------------|
| L-TRP      | 263 ± 36         | 795 ± 110***    |
| QUIN       | 9.41 ± 0.91      | 7.00 ± 0.55*    |
| QUIN/L-TRP | 20.8 ± 3.7       | 4.80 ± 0.62**   |

All values are means ± SEM (uncorrected for probe recovery). Values in the PCS group significantly different from the sham group are indicated as

\* $p < .05$ ;

\*\* $p < .01$ , and

\*\*\* $p < .001$  (Student's *t*-test).



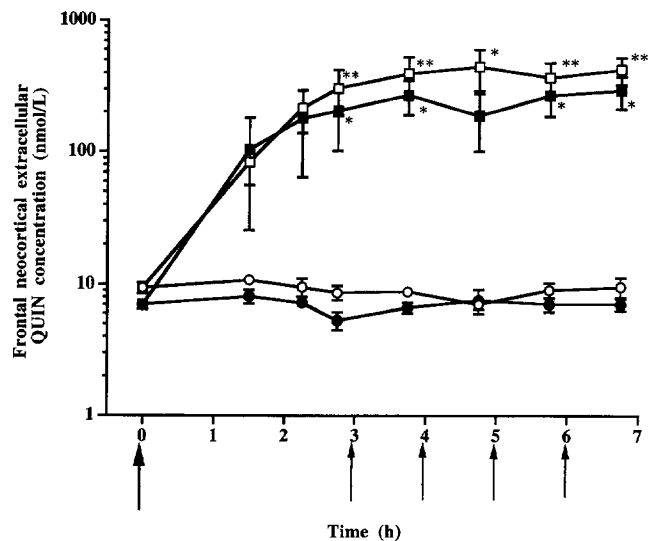
**Figure 1.** The brain extracellular L-TRP during a 6.5-hour period of continuous neocortical dialysis following challenges of L-TRP or saline to PCS and sham-operated rats. *Thick arrow*, administration of a single loading dose of L-TRP (280 mg/kg body weight I.P.), *thin arrows*, four consecutive injections of lower L-TRP doses (50 mg/kg I.P.). *Open circles*, sham-saline; *open squares*, sham-L-TRP; *solid circles*, PCS-saline; *solid squares*, PCS-L-TRP. All values are means  $\pm$  SEM and expressed as nanomoles per liter (uncorrected for probe recovery). \*, Significant ( $p < .05$ ) difference compared to respective basal value within each group (ANOVA). † and †††, Significant ( $p < .05$  and  $0.001$ , respectively) differences between PCS-L-TRP and sham-L-TRP (ANOVA).

ward lower L-TRP levels was proven to be significant from 3.5 hours and onward for sham-saline and from 4.5 hours onward for the PCS-saline rats. However, 24 hours after the start of dialysis these L-TRP values had returned to  $415 \pm 270$  nmol/L for sham-saline ( $n = 3$ ) and  $1310 \pm 910$  nmol/L for PCS-saline ( $n = 3$ ). These latter values are not shown in Figure 1, but the data were not found to be statistically different from those obtained as basal values before the challenge. In order to obtain these data, the dialysis probe had to be kept in place overnight. Because of some difficulties for dialysis probe patency during this extended period of time, not all animals could present dialysates after 24 hours of a technical quality acceptable for L-TRP analysis, which thereby increased the variability of the results within each group.

As early as 75 minutes after the initial L-TRP challenge, the sham-L-TRP and PCS-L-TRP groups displayed both significantly ( $p < .05$ ) higher L-TRP levels (approximately 14- and 8-fold) than baseline values for the groups, respectively (squares in Figure 1). The L-TRP level thereafter diminished continuously in the sham-L-TRP rats despite the addition of successive lower doses of L-TRP during the 6.5-hour course of dialysis.

Conversely, for the PCS-L-TRP rats the elevated concentrations of L-TRP following the challenges remained at about the same level during the whole 6.5-hour period of dialysis. Accordingly, differences in time course of the extracellular L-TRP between sham and PCS following this procedure of exogenous L-TRP challenge were evident; this is shown in Figure 1 as significantly higher L-TRP levels in the PCS-L-TRP versus sham-L-TRP following 3.5 hours of the challenge ( $p < .05$ ) and sustained at 4.5, 5.5, and 6.5 hours, respectively ( $p < .001$ ). At the 24-hour time point the L-TRP levels had returned to  $790 \pm 530$  nmol/L for sham-L-TRP ( $n = 3$ ) and  $1,040 \pm 690$  nmol/L for PCS-L-TRP ( $n = 2$ ). None of these values were found to be statistically different from those obtained prior to the L-TRP challenge.

Increases in L-TRP availability led to higher levels of brain extracellular QUIN to a comparable degree in both the sham-L-TRP and PCS-L-TRP groups (squares in Figure 2). The initial loading dose of L-TRP resulted in significantly higher QUIN levels at 2.5 hours and onward following the loading L-TRP challenge than basal value in both sham and PCS rats. The subsequent four successive doses of L-TRP resulted in significantly elevated steady-state QUIN levels as compared to the basal values in all rats subjected to this treatment. These steady-state QUIN levels in the PCS-L-TRP and sham-



**Figure 2.** The brain extracellular QUIN levels during a 6.5-hour period of continuous neocortical dialysis following challenges of L-TRP or saline to PCS and sham-operated rats. *Thick arrow*, administration of a single loading dose of L-TRP (280 mg/kg body weight I.P.); *thin arrows*, four consecutive injections of lower L-TRP doses (50 mg/kg I.P.); *open circles*, sham-saline; *open squares*, sham-L-TRP; *solid circles*, PCS-saline; *solid squares*, PCS-L-TRP. All values are means  $\pm$  SEM and expressed as nanomoles per liter (uncorrected for probe recovery). \* and \*\*, Significant ( $p < .05$  and  $.01$ , respectively) differences compared to respective basal value within each group (ANOVA).

L-TRP groups were about 30- and 20-fold higher than the respective basal QUIN values and were both equivalent to QUIN levels of about 200 nmol/L. Although statistically nonsignificant, the area under curve (AUC) values were about 30% lower for the PCS-L-TRP rats than for the sham-L-TRP animals ( $77.1 \pm 7.7 \mu\text{mol/L} \times \text{minute}$  versus  $107 \pm 33 \mu\text{mol/L} \times \text{minute}$ ), indicating a generally lower accumulation of QUIN following L-TRP administration in the PCS rats. The administration of saline did not significantly affect the QUIN levels either in the sham-saline or in PCS-saline groups.

## DISCUSSION

It has been suggested that the endogenous NMDA-receptor agonist QUIN plays a role in several central nervous system (CNS) diseases, including epilepsy, the neuropathology frequently associated with acquired immunodeficiency syndrome, Huntington's disease, as well as in HE (for an overview, see Stone 1993). In more detail, elevated brain QUIN levels in humans with HE as well as in PCS and acute hyperammonemic rats led to a hypothesis that increased L-TRP availability in the brain may subsequently increase brain QUIN levels and that elevated brain QUIN levels could contribute to the neurological alterations observed in HE (Moroni et al. 1986a, 1986b). More recent data, however, do not support the contention of a major role for QUIN in the pathogenesis of HE (Basile et al. 1995a, 1995b; Bergqvist et al. 1995a). The previous studies assessing brain QUIN in the development of HE have, however, addressed this issue by investigating only brain tissue levels of this potential neurotoxin. Based on the fact that QUIN is a potent competitive NMDA-receptor agonist *in vivo* the present study was designed to provide necessary complementary information on QUIN levels in the brain extracellular fluid in chronic HE.

The present study reports two novel findings concerning brain QUIN in experimental chronic HE. First, basal frontal neocortical extracellular levels of QUIN at 5 to 6 weeks of chronic PCS were significantly lower than those for sham-operated controls. These decreased (by 26%) basal frontal neocortical extracellular fluid QUIN levels are in agreement with the reduced (by 27%) basal neocortical tissue QUIN levels also seen in chronic PCS rats (Bergqvist et al. 1995a). Hence, chronic HE is probably not associated with increases either in neocortical brain tissue or extracellular fluid QUIN concentrations. The second finding relates to the observation of a rather similar accumulation profile for brain extracellular QUIN in both PCS and sham rats following increased availability to L-TRP. The only difference in brain extracellular QUIN observed between sham and PCS rats following pharmacological manipulation of the L-TRP availability was a nonsignificant decrease

in the AUC for the QUIN accumulation in the PCS rats compared with the sham-operated controls. The parallelism between PCS and sham-operated rats is rather unexpected in view of previous reports of significant alterations of L-TRP metabolism following hepatic failure in human (Rössle et al. 1986) and rats (Bengtsson et al. 1991).

Previous studies have shown that both blood QUIN levels (Heyes and Markey 1988b), as well as tissue (Moroni et al. 1984; Heyes and Markey 1988b) and extracellular (During et al. 1989a, 1989b) brain QUIN levels, rise significantly following increased L-TRP availability. The time course for the brain extracellular QUIN changes displayed in both PCS and control rats showed an early rapid increase following the first loading dose of L-TRP. About 3 hours after the loading dose the QUIN levels reached a peak level at about 20 to 30 times that of the basal levels. The subsequent lower doses (50 mg/kg) of L-TRP resulted in the achievement of a plateau level of similar degree in both PCS and sham. This phenomenon could indicate either a saturation of the efflux systems carrying QUIN out from the CNS or, perhaps more probable, a saturated QUIN metabolism in the brain. The enzyme responsible for the catabolism of QUIN in the brain is quinolinic acid phosphoribosyltransferase (E.C. 2.4.2.19; QPRT). In the rat brain, the maximal velocity of reaction for QPRT has been demonstrated to be relatively low (Speciale et al. 1987). Hence, it can be speculated that brain QPRT activity may be saturated after excess exposure to QUIN. In this context it should also be pointed out that the enzyme responsible for the major source of kynurenine production in the body is hepatic tryptophan-2,3-dioxygenase (E.C. 1.13.11.11). Several studies have demonstrated that the QUIN found in the brain under normal conditions probably results from a peripheral QUIN production in the liver followed by a passive diffusion (Kitt and Spector 1987) of QUIN in the blood across the blood-brain barrier (BBB) into the brain (Heyes et al. 1993, see also Bergqvist et al. 1995a). In normal conditions (e.g., without brain inflammation), no direct conversion of L-TRP into QUIN has been demonstrated to occur (Heyes et al. 1993). Hence, in rats with experimental hepatic dysfunction (i.e., the PCS rats) it could be anticipated that peripheral QUIN production is lower than in the sham-operated controls, and a possible consequence of this should be that the brain levels of QUIN would be lower after PCS than under normal circumstances. Accordingly, this hypothesis was supported by the findings in both the present investigation and a recent report (Bergqvist et al. 1995a). However, whether a decreased brain QUIN level associated with PCS in the rat is solely due to hepatic dysfunction *per se* or whether some other factor(s) may also come into play cannot be determined at present. Future investigations on this matter are clearly warranted. It should also be pointed out that the QUIN precursor kynurenine can

cross the BBB and that enzymes converting kynurenine to QUIN are indeed present in the brain (Heyes et al. 1993). Accordingly, the elevated brain extracellular QUIN levels following elevated L-TRP availability seen in the present study might not only be accounted for by an increased peripheral QUIN production and diffusion over the BBB, but a possibly increased peripheral kynurenine formation with a subsequently increased BBB passage of kynurenine followed by the enzymatic conversion into QUIN also has to be taken into account.

The present study shows that there is no major qualitative difference with regard to QUIN levels between PCS rats and sham-operated controls in the immediate response to a L-TRP load. Levels of QUIN of around 100 nmol/L have been demonstrated to cause focal degeneration to corticostriatal cell cultures *in vitro* (Whetsell and Schwarcz 1989). The QUIN values displayed both by the sham and PCS rats following the L-TRP provocation (approximately 200 nmol/L) are thus above this level of neurotoxicity. The L-TRP challenge utilized in the current investigation was, however, not aiming at the possible neurotoxic effects of QUIN following the provocation in the short- as well as long-term perspective but, rather, a description of the immediate neurochemical events that could follow such a L-TRP load. Further studies specifically investigating possible neurotoxic effects of QUIN following provocation with L-TRP are thus warranted in the future. Moreover, a more intricate delineation of exogenous systemic L-TRP effects on brain L-TRP and QUIN turnover would necessitate a broader procedure of varying single doses of administered L-TRP and also of repeated dosing of L-TRP given to PCS as well as sham-operated controls. Intravenous systemic versus oral challenges of L-TRP administration also should be undertaken to define aspects such as possible differences between bioavailability in the PCS group and in controls.

In the control rats of the present study, brain extracellular L-TRP concentrations peaked about 1 hour after L-TRP administration, which is consistent with previous reports from a similar challenge (Hutson et al. 1985; Carboni et al. 1989; Carboni and Di Chiara 1989), although in these reports the dosage regimens of L-TRP were slightly different from those in the present study. In the L-TRP-challenged PCS rats in the present study the L-TRP levels reached a peak value within a similar time frame as the L-TRP-challenged sham rats. This value for the PCS rats, however, remained constant throughout the L-TRP challenge experiment. This different time course may be explained by the fact that PCS rats already exhibit elevated brain extracellular L-TRP levels before L-TRP challenge, possibly because of an impaired liver L-TRP metabolism. The ability to metabolize L-TRP has, for example, been demonstrated to be lower in cirrhotic patients (Rössle et al. 1986). Accordingly, following a superimposed L-TRP load to PCS

rats, plasma L-TRP levels might significantly increase and the transport of L-TRP from the blood to the CNS might hence be saturated. Although the plasma L-TRP levels are further increased after the L-TRP challenges, the transport of L-TRP into the brain might be kept on a constant level and levels of brain extracellular L-TRP do therefore not increase any further in the L-TRP-challenged PCS rats.

The progressively declining brain extracellular L-TRP levels during the experiments in the saline-administered rats may be due to a drainagelike effect of the probe or, possibly, to normal well-known diurnal variations appearing in the brain for this aromatic amino acid. For example, previous studies have shown that the brain tissue L-TRP content decreases during the light period of the day and increases during the night (Crandall and Fernstrom 1981).

In summary, the present study demonstrates significantly lower basal brain extracellular fluid QUIN levels in chronic PCS rats than in sham-operated controls. Administration of the precursor amino acid L-TRP significantly elevated the QUIN levels in both sham and PCS rats compared to their respective basal QUIN levels. No clear qualitative difference in the QUIN response to this change in L-TRP availability could, however, be seen between sham and PCS rats. We conclude that chronic experimental HE is not associated with an increased brain extracellular QUIN concentration. These findings, in conjunction with our previous results, do thereby not support a theory of involvement of QUIN in the development of HE.

## ACKNOWLEDGMENTS

The authors are grateful to Kerstin Haraldsson and Anna-Karin Oldén for their skillful technical assistance. We also thank Professor Anders Björklund at the Department of Medical Cell Research, Lund University, Lund, Sweden, for providing space and equipment for the microdialysis experiments.

The generous financial support by the Swedish Society of Medicine, the Åke Wibergs Foundation (Stockholm, Sweden), the Medical Faculty of Lund University, the Crafoordska Foundation, the Anna-Lisa and Sven-Eric Lundgrens Foundation for Medical Research, the Torsten and Elsa Segerfalks Foundation, the Maggie Stephens Foundation, Magnus Bergvalls Foundation, Fredrik and Ingrid Thuring's Foundation, and the Royal Physiographic Society (Lund, Sweden) is gratefully acknowledged.

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