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THE PHYSIOPATHOLOGICAL ROLE OF QUINOLINIC ACID IN THE MAMMALIAN CENTRAL NERVOUS SYSTEM

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Summary. – *The content of the supposed tryptophan (TRY) metabolite, quinolinic acid (QUIN), has been evaluated using a mass-fragmentographic method, in the CNS of rats and humans, in situations characterized by an increased or a decreased availability of TRY to the brain. The cortical and cerebellar content of QUIN increased in a dose-dependent manner when TRY (20-200 mg/kg) was administered i.p. to rats. Similar results were obtained when TRY liver metabolism was decreased. This occurred both in rats and humans. In fact rats bearing a porto-caval anastomosis had increased concentration of QUIN in their cortex and the content of QUIN was 3 times higher in the cortex taken from patients who died after hepatic coma than in controls. Similar results were obtained in the CSF of patients affected by liver disease. Our results suggest that QUIN could be one of the toxins involved in the pathogenesis of brain disorders, associated with liver pathology. In rats, an unexpected increase of QUIN content was also found after a chronic TRY deprivation. The possibility that brain QUIN may also be synthetized through TRY-indipendent pathways is discussed.*

Riassunto (Il ruolo fisiopatologico dell'acido chinolinico nel sistema nervoso centrale dei mammiferi). – *Scopo della nostra ricerca è stato quello di valutare il ruolo fisiopatologico dell'acido chinolinico, una eccitotossina finora considerata un metabolita inerte del triptofano. Per questo abbiamo misurato con una tecnica di frammentografia di massa le variazioni del contenuto corticale di questa molecola in condizioni caratterizzate da un aumento o da una diminuzione della disponibilità dell'aminoacido precursore. Un aumento della concentrazione di acido chinolinico è stato osservato sia dopo somministrazione i.p. di TRY che dopo diminuzione del suo metabolismo epatico. Ratti con anastomosi porto-cava e pazienti con funzionalità epatica compromessa hanno concentrazioni elevate di questa eccitotossina sia nel cervello che nel liquido cefalorachidiano. Ciò ha fatto ipotizzare che l'acido chinolinico possa essere una delle tossine coinvolte nella patogenesi dei sintomi neurologici associati a malattie epatiche. Sorprendentemente l'acido chinolinico si accumula anche nel S.N.C. di ratti nutriti per 15 giorni con una dieta carente di triptofano. La possibilità che questa molecola possa essere sintetizzata anche attraverso vie metaboliche indipendenti dal TRY è stata posta in discussione.*

Introduction

Quinolinic acid (QUIN) is a tryptophan metabolite, able to cause seizures in mice, frogs and rats [1-3] when it is injected intracerebroventricularly. It excites rat cortical neurons [4, 5] when microiontophoretically applied.

These excitatory effects are antagonized by AP5 (aminophosphonovaleric acid) at doses which do not affect quisqualate or kainate responses. This implies that in the rat cortex QUIN is an agonist of the NMDA receptors [4, 6]. Finally, intrahippocampal or intrastriatal infusions of QUIN cause axon-sparing neuronal destruction reminescent of that caused by kainic and ibotenic acids [7]. We have recently demonstrated that QUIN is present in the mammalian brain [8] and that its concentration changes during the aging process [9]. In the present study we investigated which are the mechanisms regulating QUIN synthesis and whether or not changes of its content can be associated with pathological conditions.

Methods

Animals. – The experiments here reported were performed in male Wistar rats (150-200 g) fed *ad libitum* until experiments.

The animals were decapitated and the brain quickly removed and dissected.

Autopsy material and patients. – All autopsy material was obtained from the Montreal General

Hospital. The time interval between death and refrigeration of cadavers was approximately 2 h. The autopsies were performed within 6-24 h after death. Half of the brain was immediately placed in dry ice and stored at −80° C.

Cerebro spinal fluid (CSF) was obtained from patients with liver cirrhosis (confirmed by needle biopsy) during an episode of hepatic coma. Control CSF was obtained from patients admitted to the neurological clinic for diagnostic purposes; none was in coma.

Mass-fragmentographic measurement of QUIN. – In our laboratory a mass-fragmentographic approach for measuring QUIN has been widely used since 1983 [8, 10]. Comparable results were obtained by other authors [11]. Recently using this method we showed that QUIN is present in measurable amount in the human CSF as well [12].

Details of the extraction and detection procedure of QUIN are herein reported. Brain areas (approximately 250-500 mg of fresh tissue) are homogenized in 2 ml of 80% ethanol containing 100 µl of 0.5 M NaOH and a known amount (0.5 - 2 nmol) of 2,4-pyridindicarboxylic acid as an internal standard. After a first centrifugation (6000 *g*; 15 min), the pellet is again resuspended and extracted in 2 ml 80% ethanol. The collected supernatants are placed overnight at −80° C to precipitate fatty materials which are discarded. An ion exchange resin, Dowex AGI WX8 (100-200 mesh formeate form, 250 mg) is subsequently added to the supernatants in order to separate QUIN and its isomer (the internal standard) from interfering materials. Each sample is carefully mixed for at least 5 min and then centrifuged in order to separate the material absorbed to the resin from the rest of the supernatant. This procedure is repeated in order to wash the resin with O.1M NaOH (two times), with water (three times) and with O.1M formic acid (two times).

The elution of QUIN and of its internal standard from the resin is obtained using 5 M formic acid (1 ml four times). The eluate is then dried in a vacuum centrifuge and the dry residue is resuspended in ethanol (50%, 400 µl) containing 0.05 M KOH (20 µl). Particular care has to be used in the resuspension step since QUIN is poorly soluble if ethanol is acidic. This may occur when formic acid is not completely evaporated. The pH of each sample must therefore be checked and adjusted to 7.5-8.5.

In separate experiments we have calculated that radiolabelled QUIN added to brain homogenates and passed through the entire purification procedure is recovered for 80-90%. The resuspension mixture is placed in Kontes vials and evaporated to dryness. To the dry residue, 50 µl of hexafluoroisopropanol (Pierce) and 50 µl of freshly open, water free, trifluoroacetic anhydride are added in order to esterify the carboxylic groups of QUIN. The vials are capped and heated at 60° C for 1 h. The reagent mixture is then evaporated under nitrogen and the residue is dissolved in 12 µl of acetone. Two microliter aliquots of this solution are then injected into a gas-chromatograph-mass spectrometer (LKB - 2091).

The gas-chromatographic conditions used are: sylanized glass column (2.5 m × 2 mm) containing 5% OV 17 on Chromosorb, 100-200 mesh; helium flow rate 12-16 ml/min; oven temperature 140-160° C; flash heater temperature 180-200° C; separator temperature 220-240° C.

The mass-spectrometric conditions used are: electron energy 60 eV; acceleration voltage 3.5 KV; instrument resolving power 1000.

Synthetic QUIN, run through this procedure and injected into the GC-MS gives a single and sharp gas-chromatographic peak and the gas-chromatographic properties are shown in Table 1. Four fragments can be used to identify QUIN. They are: 1) the molecular ion (467); 2) a fragment having an m/z of 448 (molecular ion minus a fluoride); 3) a third one having an m/z of 300 (molecular ion minus the esterifying group -O-CH-C2-F6) and, 4) a fragment (which is due to the loss of one esterified carboxylic group of the derivatized molecule) having an m/z 272. This fragment is the basal peak and has been routinely used for our quantitative measurements of single ion monitoring.

The above described approach allows us to quantify QUIN in the brain and has a sensitivity limit of approximately 10 picomol of QUIN per injection. A minor modification of the method can be used to identify and measure QUIN in the cerebrospinal fluid.

The modification consists in 3 ml of absolute ethanol and 200 µl of NaOH 0.5 N containing 0.5-1 nmol of 2.4 pyridinedicarboxylic acid are added to a known volume of CSF (3-5 ml). The mixture is stored overnight at −50° C to precipitate proteins and fatty material.

All the subsequent steps are identical to those previously described. Table 1 reports that synthetic

Table 1. – *Identification of quinolinic acid in the rat cortex and in human CSF*

	Retention time	Peak height ratio	
		272/300	448/467
Synthetic compound	3 min 43 s ± 2 s	1.5 ± 0.1	9.9 ± 0.2
Material extracted from rat brain	3 min 45 s ± 2 s	1.5 ± 0.2	10.1 ± 0.2
Material present in human CSF	3 min 45 s ± 1 s	1.5 ± 0.1	10 ± 0.2

The number 272, 300, 448 and 467 are the m/z of four fragments of derivatized quinolinic acid (see the formula and the text). The fragmentation of the molecule was obtained under the electron inpact mode (70 eV); instrument resolving power 500; accelerating voltage 3.5 kV; ion source temperature 240° C. The chromatographic conditions used were: sylanized glass column containing OV-17 5% on Chromosorb 100-120 mesh (2 m × 0.2 mm); helium flow 15 ml/min; oven temperature 145° C; flash heater temperature 190° C.

QUIN and the material we are measuring in human CSF have the same fragmentation pattern and the same chromatographic properties thus indicating that QUIN is present in the CSF as well.

Measurements of 5HT and 5HIAA using high performance liquid chromatography with an electrochemical detector (LCED). – Various brain areas were homogenized in cold 0.2M $HClO_4$, containing 0.01% EDTA. The supernatant was filtered and injected in a LCED apparatus. The filtration was performed according to the procedure described by Van Valkenburg *et al.* [13]. The LCED apparatus consisted of a Perkin Elmer Mod 10 A pump, a Reodyne 7125 injector equipped with a 50 µl sample loop, an RP8 guard column, a reverse-phase column (Perkin-Elmer HS-5C8) and an electrochemical detector (LCA 15 EDT London) operated at + 0.65 V. The mobile phase was selected according to Kilts *et al.* [14] and consisted in a solution (pH 2.5) containing Na_2HPO_4 75 mM, citric acid 100 mM, methanol 10%, sodium heptansulfonate 0.9 mM.

Results and discussion

The distribution of QUIN in the mammalian CNS. – The content of QUIN in the human cortex, in the cerebrospinal fluid and its distribution in the brain of the rat, guinea pig and rabbit are reported in Table 2. In spite of the fact that the concentrations of QUIN in the brain do not vary greatly between different regions and are approximately in the micromolar range, the cortex constantly contains the highest concentration and the striatum the lowest. The concentration of QUIN in human CSF is approximately 2.10^{-8} M and it is therefore relatively distant from that known to exert excitotoxic lesions ($= 10^{-5}$ M or 500 times higher). However, this does not rule out a possible interaction of the molecule with excitatory receptors under both physiological and pathological conditions.

Modification of brain QUIN concentration. – The relationships between tryptophan availability and the brain content of QUIN have been studied by both increasing the blood tryptophan concentration (administration of tryptophan, decrease of liver metabolism) or by decreasing it (feeding the animals with a tryptophan-free diet).

When tryptophan was administered to the rats, the brain content of both 5 HT and of QUIN increased in a dose-dependent manner, supporting the concept that the brain concentrations of these molecules are in some way dependent upon tryptophan availability. This holds true also when the availability of tryptophan to the brain is increased by decreasing its metabolism in the liver. Fig. 1 shows the content of both QUIN and 5 HT in several brain areas of rats bearing an anastomosis between the porta and the cava veins. In these rats the portal blood by-passes the liver and the blood and brain tryptophan concentrations are chronically increased [15, 16]. These findings could suggest that changes in the brain concentration of tryptophan metabolites (including 5 HT and QUIN) could be in some way involved in the changes of the CNS functions associated with liver disease.

It is certainly true that an acute or a chronic increase of blood tryptophan concentration causes a parallel shift in the concentration of its metabolites in the brain. However, surprisingly a chronic decrease of the availability of this amino acid to the brain does not result in a parallel decrease of QUIN and of 5 HT content. Fig. 2 shows that by feeding rats for 15 days with a diet lacking tryptophan and niacin the cortical content of 5 HT and of 5 HIAA decreases by 60-70% while that of QUIN slightly increases. It therefore seems that a metabolic pathway, not related to tryptophan and leading to QUIN synthesis could be present in the brain. Metabolic pathways leading to QUIN synthesis from aspartic acid, acetate and formate or from dihydroxyacetonephosphate and aspartate have been described in bacteria, in plants and in marine shrimp [17-19]. The possibility that the mammalian brain could syntheti-

Table 2. – *The brain content of QUIN*

	Rat	Guinea-pig	Rabbit	Human
Cortex	1.6 ± 0.2	1.8 ± 0.2	1.5 ± 0.2	0.6 ± 0.15
Hippocampus	1.0 ± 0.2	0.8 ± 0.2	0.5 ± 0.2	—
Striatum	0.6 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	—
Cerebellum	1.3 ± 0.2	1.4 ± 0.2	1.1 ± 0.2	—
CSF	—	—	—	22.0 ± 2 picomol/ml

Values are nmol/g. w. wt. and are the means ± SE of at least 7 determinations.

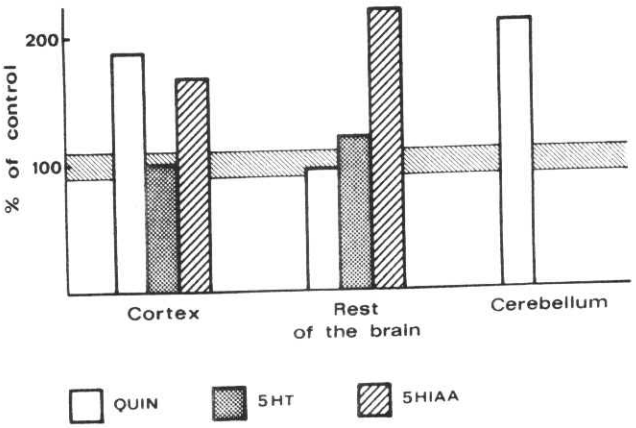

Fig. 1. – The effects of a chronic increase of blood tryptophan concentrations obtained by anastomosing the porta with the cava veins, on the brain's content of QUIN, 5-HT and 5HIAA.

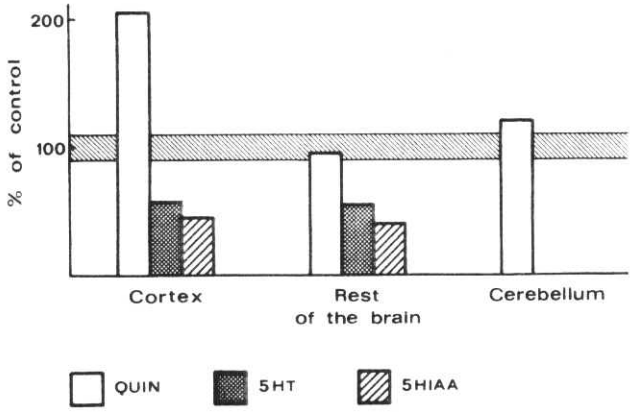

Fig. 2 - Differential effects of a tryptophan-free diet on the cortical content of 5-HT, 5HIAA and of QUIN.

ze QUIN independently from tryptophan availability deserves further attention. An activation of metabolic pathways leading to the synthesis of QUIN independently from tryptophan availability could cause an imbalance between the actions of several electrophysiologically active tryptophan metabolites. In particular, we think it extremely important to measure the content of kynurenic acid under these conditions. In this context, it is probably worthwhile to remember that a tryptophan-free diet may cause pellagra, a disease characterized by symptoms affecting the CNS such as allucinations and dementia.

Ontogenetic studies. – The number of neurons in the cortex usually decreases in old age [20] and with the aim of clarifying whether or not QUIN could play a role in the neuronal degeneration which takes place during the aging process, we measured QUIN concentrations in the cortex of rats aged 3 days, 3 months, 9 months and 30 months.

The cortical content of QUIN increased during the maturation and aging. In the group of 30 month old animals a large variability of QUIN content was observed and at least 5 out of 12 rats had extremely high (5-9 nmol/g wet weight) cortical concentrations of QUIN (Tab. 3).

We have no information on the possibility that this subpopulation of aged rats shows QUIN-related morphological changes in brain. However, our data could be in agreement with the hypothesis that an accumulation of QUIN could be one of the factors involved in the dropout of neurons and synapses which may occur during the aging process of the rat [20, 21].

Human studies. – Our studies on the measurement of the content of QUIN in the rat brain suggest that an abnormal accumulation of this tryptophan metabolite could occur in at least two widely studied human pathological situations. The first one is the loss of neurons leading to dementia which may occur in old age, possibly the senile dementia Alzheimer type (SDAT). The second is abnormal neuronal function associated with an increased tryptophan availability to the brain and due to an impaired utilization and metabolism of this essential amino acid in the liver. In preliminary investigations we showed that after death, under the conditions used in most hospitals to store cadavers, the brain concentration of QUIN is relatively stable. This allowed us to verify our hypothesis by measuring the content of this excitotoxin in autopsied samples. We obtained from the brain bank of the Douglas Hospital Research Centre (Verdun - Canada) the frontal, parietal and temporal cortex of 8 patients who died after a clinical and histopathological diagnosis of SDAT and the same brain areas of 7 cadavers died after different pathologies. Every SDAT patient had a reduced number of neurons in its cortex and also in the nucleus basalis magnocellulars. Furthermore, clear morphological signs of Alzheimer changes (plaques and fibrillary tangles) were present in the hippocampus, frontal and parietal cortex. In spite of this, no changes of the QUIN content in the 3 cortical areas examined was present (Table 3).

Thus, in this neurodegenerative disorder, the loss of cell bodies in the cortex does not seem to be associated with changes of the steady state level of QUIN.

Unfortunately in human studies it is difficult to rule out possible transient changes of the content and of the turnover of the toxin as a possible pathogenetic factor. Nevertheless, on the basis of steady state content, our data do not support the possibility that an accumulation of QUIN plays an important role in the neuronal loss typical of the SDAT. The increased brain content of QUIN in aged rats could suggest a role for the toxin in the physiological aging process. However, we have no information on the matter because a systematic study on the content of QUIN in the human cortex of different ages has not been performed.

The second human pathological situation we studied was hepatic coma. The data reported in Table 4 show that the frontal cortex of patients who

Table 3. – *QUIN content in the parietal cortex of rats at different ages and in three areas of autoptical human cortex*

Rats		Age		
		3 months	12 months	20 months
QUIN nmlo/g w.w.		1.6 ± 0.6	2.1 ± 0.3	4.5 ± 0.9
Humans			Control Age 70 ± 6	Alzheimer Age 74 ± 7
QUIN nmol/g w.w.	Frontal		0.5 ± 0.15	0.4 ± 0.1
	Temporal		0.5 ± 0.1	0.4 ± 0.1
	Parietal		0.6 ± 0.15	0.7 ± 0.3

died after hepatic coma contains concentrations of QUIN 2-3 times higher than that of controls. Furthermore, the concentration of QUIN in the CSF of control patients affected by different pathologies was 22 ± 7 picomol/ml while during hepatic coma the CSF QUIN content increased six times.

These findings allow us to apply to man similar data obtained in rats bearing a porto-caval anastomosis (see Fig. 1). Therefore, they support the concept that QUIN should be added to the list of compounds involved in the pathogenesis and symptomatology of brain disorders associated with liver failure. It is a common clinical observation that a meal containing large amounts of tryptophan (such as a meat meal) causes a worsening of the encephalopatic symptomatology in patients affected by chronic liver failure. The increased availability of the amino acid could cause an increased synthesis of QUIN. However, the importance of these changes in the overall pathogenesis and in the symptomatology of hepatic coma is not yet clear. In our opinion, important progress in the field could be expected from studies aimed at better understanding the role of tryptophan metabolites (including QUIN and Kyn) in the brain functions. A clarification of their role and their receptors in human CNS would certainly be helpful.

Table 4. – *The content of QUIN in the frontal cortex and in the CSF of humans affected by hepatic encephalopaty*

	QUIN nmol/g	
	Frontal cortex	CSF
Control	0.8 ± 0.1	0.02 ± 0.002
Hepatic encephalopaty	2.55 ± 0.6	0.14 ± 0.05

Conclusions

Using a mass-fragmentographic method it has been demonstrated that QUIN is present in the mammalian brain and in the CSF. It is possible to increase the brain content of QUIN by giving large amounts of tryptophan to the animals or by decreasing its liver metabolism. This has been demonstrated in the brain of patients who died after hepatic coma as well. Free QUIN has also been demonstrated in human CSF and the finding that its content increases in patients affected by liver disease allows us to consider QUIN as one of the toxins contributing to the symptomatology of hepatic encephalopathy [22]. On the other hand, it is not possible to decrease the brain content of QUIN by feeding animals with a tryptophan-free diet. The possibility that metabolic pathways not related with tryptophan may lead to QUIN synthesis similarly to what occurs in plants or in bacteria should be considered. This could be important in view of the opposite electrophysiological action of QUIN and Kynurenic acid [4]. Since both of them are synthetized from tryptophan under physiological conditions, when QUIN start to be sinthetized through different metabolic pathways it is possible to predict situations characterized by an imbalance between the two systems. The pathological brain changes occurring in patients affected by pellagra could find a biochemical explanation in the loss of equilibrium between the availability of QUIN and Kyn.

Finally, we showed that in rats aged 30 months QUIN content largely increases when compared to younger rats, while no differences were found in the cortical content of QUIN in patients died after SDAT when compared to those affected by other disorders. It is therefore difficult that an accumulation of QUIN could play a role in this type of dementia, but an accumulation of the molecule could be one of the factors involved in the "physiological" aging process.

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