Tricarboxylic Acid Cycle Activity Measured by $^{13}$C Magnetic Resonance Spectroscopy in Rats Subjected to the Kaolin Model of Obstructed Hydrocephalus

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Abstract Evaluating early changes in cerebral metabolism in hydrocephalus can help in the decision making and the timing of surgical intervention. This study was aimed at examining the tricarboxylic acid (TCA) cycle rate and $^{13}$C label incorporation into neurotransmitter amino acids and other compounds 2 weeks after rats were subjected to kaolin-induced progressive hydrocephalus. In vivo and ex vivo magnetic resonance spectroscopy (MRS), combined with the infusion of [1,6-$^{13}$C]glucose, was used to monitor the time courses of $^{13}$C label incorporation into the different carbon positions of glutamate in the forebrains of rats with hydrocephalus as well as in those of controls. Metabolic rates were determined by fitting the measured data into a one-compartment metabolic model. The TCA cycle rate was $1.3 \pm 0.2$ µmoles/gram/minute in the controls and $0.8 \pm 0.4$ µmoles/gram/minute in the acute hydrocephalus group, the exchange rate between α-keto-glutarate and glutamate was $4.1 \pm 2.5$ µmoles/gram/minute in the controls and $2.7 \pm 2.6$ µmoles/gram/minute in the hydrocephalus group calculated from in vivo MRS. There were no statistically significant differences between these rates. Hydrocephalus caused a decrease in the amounts of glutamate, alanine and taurine. In addition, the concentration of the neuronal marker N-acetyl aspartate was decreased. $^{13}$C Labelling of most amino acids derived from [1,6-$^{13}$C]glucose was unchanged 2 weeks after hydrocephalus induction. The only indication of astrocyte impairment was the decreased $^{13}$C enrichment in glutamine C-2. This study shows that hydrocephalus causes subtle but significant alterations in neuronal metabolism already early in the course of the disease. These sub-lethal changes, however, if maintained and if ongoing might explain the delayed and programmed neuronal damage as seen in chronic hydrocephalus.

Keywords In vivo $^{13}$C MR spectroscopy · Astrocytes · Neurons · Glucose · Metabolism

Abbreviations
CSF Cerebrospinal fluid
HPLC High performance liquid chromatography
MRI Magnetic resonance imaging
MRS Magnetic resonance spectroscopy
NAA N-acetyl aspartate
TCA Tricarboxylic acid

Introduction

In hydrocephalus the intracranial cerebrospinal fluid (CSF) volume is increased followed by compression of brain tissue. Kaolin-induced hydrocephalus is an intensively...
studied model of progressive hydrocephalus first described in 1932 [1]. In this model kaolin is injected under microscopic guidance into the cisterna magna resulting in a sterile meningeal inflammation. This leads to an obstruction of the foramina of Luschka and Magendi and thus to hydrocephalus and syringomyelia [2]. Clinical symptoms of acute hydrocephalus are evoked by an increase in intracranial pressure and outflow resistance. Treatment of chronic hydrocephalus is operative, but decision making concerning the appropriate timing of surgical treatment is difficult. Despite enormous efforts in hydrocephalus research, this issue is still not satisfactorily solved. Thus, non-invasive techniques to assess development of hydrocephalus have to be established. A possible solution might be to monitor changes in cerebral metabolism which has been shown to take place in hydrocephalus [3]. In vivo MRS can provide valuable information about cerebral metabolites and tricarboxylic acid (TCA) cycle rates and thus, such studies of the brain with hydrocephalus could be of significant clinical importance.

$^{13}$C MRS has been employed extensively to study cerebral metabolism and TCA cycle activity in animals after the injection of $^{13}$C labelled substrates. Since $^{13}$C labelled metabolites such as glutamate and aspartate are in equilibrium with TCA cycle intermediates, the level of labelling of these measured metabolites reflect TCA cycle activity. The present study is the first to describe the in vivo TCA cycle rate in rats subjected to hydrocephalus. Hydrocephalus was induced by kaolin-instillation into the cisterna magna of Sprague–Dawley rats and 2 weeks later $[1,6-^{13}$C$]$glucose was infused and in vivo MRI and $^{13}$C MRS were performed. The incorporation of $^{13}$C label from $[1,6-^{13}$C$]$glucose into $[4-^{13}$C$]$- and $[3-^{13}$C$]$glutamate was thus observed. The time courses of $^{13}$C label incorporation into glutamate in the brains of rats with hydrocephalus as well as in controls were fitted using a one-compartment metabolic model allowing non-invasive determination of the neuronal TCA cycle rate and the exchange rate between $\alpha$-ketoglutarate and glutamate. Since most glutamate is located in neurons [13], metabolic rates obtained using this model primarily reflects neuronal metabolism. Thereafter, labelling of different metabolites in the cortex was measured by ex vivo $^{13}$C MRS and amino acid concentrations were determined using high performance liquid chromatography (HPLC) of tissue extracts.

### Experimental Procedures

#### Animals

All experimental procedures were approved by the Norwegian Experimental Animal Board. Animals had free access to water and food and were kept under standard laboratory conditions (room temperature $22^\circ$C, 66% humidity, 12/12 h light/dark cycle). Five Sprague–Dawley rats (2 months old, 200 g, Mollergaard Breeding Centre, Copenhagen, Denmark) were anaesthetized with ketamine (100 mg/kg, ketalar, Warner Lambert, Nordic AB, Solna, Sweden) and medetomidinehydrochlorid (0.5 mg/kg, Janssen-Cilag Ltd., Sollentuna, Sweden) and made hydrocephalic by microsurgical instillation of 0.1 ml kaolin (4 g/ml 0.9% saline, NMD, Oslo, Norway) into the cisterna magna. Five rats were sham-operated and served as controls. Postoperatively, rats were kept one per cage at a light–dark shift of 12 h and received water and food ad libitum. In vivo MRS was performed in all animals, but due to technical problems only 3 spectra of sufficient signal to noise ratio of the peaks in the $^{13}$C MR spectra were obtained for each group. In addition, 4 rats were used to determine the input function of glucose.

#### Determination of the Input Function of Glucose

The time courses of incorporation of labelling in $[4-^{13}$C$]$- and $[3-^{13}$C$]$glutamate in the brain were fitted into a one-compartment metabolic model (see section below) which yielded the rate of the TCA cycle and the exchange rate between $\alpha$-ketoglutarate and glutamate. The time courses of incorporation of $^{13}$C labelling into glutamate C-4 and C-3 depend on the kinetics of $^{13}$C labelling of glucose in the blood. The latter time course is called the input function and was determined by measuring the $^{13}$C enrichment of glucose in blood during the infusion. Without knowledge of the input function, the rate of $^{13}$C labelling of glutamate cannot be interpreted, since it is not known how quickly the $^{13}$C labelled substrate enters the system. Since blood cannot be drawn easily inside the magnet in rats with a small blood volume, additional measurements were performed on the bench using the same infusion protocol as for the MRS experiment to determine the input function and to assess arterial pH, pCO2, pO2, glucose and lactate. Four animals were fasted for 18–20 h prior to the experiment; their weight was $258 \pm 4$ g after fasting. Spontaneously breathing animals were anesthetized with 3.5% isoflurane in 30/70% O2/N2 using a small animal ventilator (Harvard Apparatus, MA, USA) in a closed chamber. During surgery and also later during glucose infusion the animals were spontaneously breathing 2% isoflurane in 30/70% O2/N2 delivered though a face mask. Body temperature was maintained using a feed-back controlled heating table connected to a rectal probe. One catheter was introduced into the right femoral vein, and one in the right femoral artery. The insertion was sprayed with lidocain (10 mg/ doses) and closed, with the catheters emerging from the middle and posterior end of the incision. Arterial blood
samples from the control animals were drawn 5 min before the start of the intravenous glucose infusion, at the start of the infusion and thereafter then 5, 10, 15, 30, 45, 60 and 80 min after the start of the infusion. Approximately 300 μl arterial blood was drawn at each time point. One hundred micro liter was used for the measurement of pH, plasma lactate and glucose as well as blood gasses using a blood gas analyzer (Siemens RapidLab 1200, Siemens Health Care, USA). Two hundred micro liter of blood was transferred to an Eppendorf tube and centrifuged at 1,000 rpm for 5 min and at 4°C. Plasma samples were extracted with 1 ml ice-cold 7% perchloric acid and centrifuged for 10 min at 4°C and 4,000g. Each sample was neutralized with 2 M KOH, centrifuged and lyophilized. The lyophilized plasma samples were dissolved in 400 μl D2O (99%, Cambridge Isotope Laboratories, Woburn, Massachusetts, USA) and analyzed with high resolution 1H NMR to determine the fractional 13C enrichment of glucose. 1H NMR spectra were acquired on a Bruker DRX500 spectrometer 11.7T, (Bruker Biospin GmbH, Rheinstetten, Germany) and analyzed with high resolution 1H NMR to determine the fractional 13C enrichment of glucose. 1H NMR spectra were acquired on a Bruker DRX500 spectrometer 11.7T, (Bruker Biospin GmbH, Rheinstetten, Germany) and analyzed with high resolution 1H NMR to determine the fractional 13C enrichment of glucose. 1H NMR spectra were acquired on a Bruker DRX500 spectrometer 11.7T, (Bruker Biospin GmbH, Rheinstetten, Germany) and analyzed with high resolution 1H NMR to determine the fractional 13C enrichment of glucose. 1H NMR spectra were acquired on a Bruker DRX500 spectrometer 11.7T, (Bruker Biospin GmbH, Rheinstetten, Germany) and analyzed with high resolution 1H NMR to determine the fractional 13C enrichment of glucose. 1H NMR spectra were acquired on a Bruker DRX500 spectrometer 11.7T, (Bruker Biospin GmbH, Rheinstetten, Germany) and analyzed with high resolution 1H NMR to determine the fractional 13C enrichment of glucose. 1H NMR spectra were acquired on a Bruker DRX500 spectrometer 11.7T, (Bruker Biospin GmbH, Rheinstetten, Germany) and analyzed with high resolution 1H NMR to determine the fractional 13C enrichment of glucose. 1H NMR spectra were acquired on a Bruker DRX500 spectrometer 11.7T, (Bruker Biospin GmbH, Rheinstetten, Germany) and analyzed with high resolution 1H NMR to determine the fractional 13C enrichment of glucose. 1H NMR spectra were acquired on a Bruker DRX500 spectrometer 11.7T, (Bruker Biospin GmbH, Rheinstetten, Germany) and analyzed with high resolution 1H NMR to determine the fractional 13C enrichment of glucose. 1H NMR spectra were acquired on a Bruker DRX500 spectrometer 11.7T, (Bruker Biospin GmbH, Rheinstetten, Germany) and analyzed with high resolution 1H NMR to determine the fractional 13C enrichment of glucose. 1H NMR spectra were acquired on a Bruker DRX500 spectrometer 11.7T, (Bruker Biospin GmbH, Rheinstetten, Germany) and analyzed with high resolution 1H NMR to determine the fractional 13C enrichment of glucose. 1H NMR spectra were acquired on a Bruker DRX500 spectrometer 11.7T, (Bruker Biospin GmbH, Rheinstetten, Germany) and analyzed with high resolution 1H NMR to determine the fractional 13C enrichment of glucose. 1H NMR spectra were acquired on a Bruker DRX500 spectrometer 11.7T, (Bruker Biospin GmbH, Rheinstetten, Germany) and analyzed with high resolution 1H NMR to determine the fractional 13C enrichment of glucose. 1H NMR spectra were acquired on a Bruker DRX500 spectrometer 11.7T, (Bruker Biospin GmbH, Rheinstetten, Germany) and analyzed with high resolution 1H NMR to determine the fractional 13C enrichment of glucose. 1H NMR spectra were acquired on a Bruker DRX500 spectrometer 11.7T, (Bruker Biospin GmbH, Rheinstetten, Germany) and analyzed with high resolution 1H NMR to determine the fractional 13C enrichment of glucose. 1H NMR spectra were acquired on a Bruker DRX500 spectrometer 11.7T, (Bruker Biospin GmbH, Rheinstetten, Germany) and analyzed with high resolution 1H NMR to determine the fractional 13C enrichment of glucose.
The dissected cortical tissue was homogenized with an ultrasound sonicator Vibra Cell sonicator (Model VCX 750, Sonics & Materials, Newtown, CT, USA) in ice-cold 0.7% perchloric acid and thereafter centrifuged for 20 min at 4°C and at 10,000g. The supernatant was stored at −20°C for HPLC analysis. The rest of the supernatant was transferred into a new tube, neutralized with 1 M KOH and centrifuged for 20 min at 4°C and at 10,000g. The supernatants were thereafter lyophilized and dissolved in 400 μl D2O (99%, Cambridge Isotope Laboratories, Woburn, Massachusetts, USA) containing 0.1% ethylene glycol as an internal reference signal and pH was adjusted to values between 6.8 and 7.0. Proton decoupled 13C MR spectra were acquired on a 11.7T Bruker DRX500 spectrometer (Bruker Biospin GmbH, Rheinstetten, Germany), and relevant peaks in the 13C spectra were quantified by integration in XWINNMR in relation to the known amount of ethylene glycol added to the sample. The following acquisition parameters were applied: 30° pulse angle, acquisition time of 1.3 s and a relaxation delay of 2.5 s. The number of scans was typically 15,000. Some spectra were also broad band decoupled only during acquisition and accompanied by a relaxation delay of 20 s, to achieve fully relaxed spectra without NOE. From several sets of spectra, correction factors were obtained and applied to the integrals of the individual peaks.

The total amounts of glutamate, glutamine, GABA, alanine and aspartate in the extracts were quantified by HPLC analysis after derivatization with o-phthaldialdehyde essentially as described by Geddes and Wood [5], using a Hewlett Packard 1100 apparatus (Agilent, Palo Alto, CA, USA).

Percent enrichment in the 13C labelled metabolites was calculated from the following formula:

\[\%\text{ enrichment} = \frac{([X-^{13}\text{C}]\text{metabolite} - 1.1\%[\text{metabolite}]) \times 100}{[\text{metabolite}]}\]

[Metabolite] represents the total amount of a given metabolite measured by HPLC. [X-13C]metabolite represent the concentration of a 13C isotopomer of a given metabolite measured by 13C MRS in ex vivo tissue extracts. This calculation was done for each peak in the ex vivo 13C spectrum obtained for each rat individually.

A t test was thereafter used to compare the means of the HPLC and ex vivo 13C MRS data (n = 5 in each group), and the statistical significance was set to \(P < 0.05\).

Metabolic Model

The time course of plasma glucose 13C enrichment and the time courses for incorporation of 13C label into [4-13C]glutamate and [3,13C]glutamate from the in vivo MRS experiment were fitted with a comprehensive mathematical metabolic model described in Henry et al. [6]. The metabolic model consists of a set of differential equations which can be solved numerically using SAAM II (The SAAM institute, Seattle, WA). Since most glutamate is located in neurons [13], metabolic fluxes obtained using this model primarily reflect neuronal metabolism. Furthermore, the concentrations of glutamate, glutamine and aspartate measured by HPLC were also fitted into the model. Based on literature values the concentration of oxaloacetate was assumed to be 1 μmol/g and that of α-ketoglutarate was assumed to be 0.5 μmol/g in brain tissue [7]. In the one-compartment model used there were four unknown parameters; (1) a rate constant describing the neuronal TCA cycle (V TCA) (2) the exchange rate between α-ketoglutarate and glutamate (V X) (3) the rate constant describing the dilution of labelled 13C lactate through exchange with unlabelled lactate (V DIL) and (4) a dilution flux to account for loss of 13C-labelled glutamine through exchange with unlabelled glutamine (V EFF) [6]. Furthermore, the model described in Henry et al. [6] assumes that \(V_{\text{Gln}} = 0.41 \times V_{\text{TCA}}\) based on previous studies [7] and since [1,6-13C]glucose was infused the rate of glycolysis \(V_{\text{GLY}}\) was assumed to be the same as \(V_{\text{TCA}}\). Each animal was modelled individually and mean values of the rate of the neuronal TCA cycle and the exchange rate between α-ketoglutarate and glutamate were then calculated for each group. The dilution flux to account for loss of 13C-labelled glutamine through exchange with unlabelled glutamine could not be determined with accuracy for the animals in the hydrocephalus group, and the rate constant describing the dilution of labelled 13C lactate through exchange with unlabelled lactate could not be determined at all since this parameter reached the lowest limit constraint in the model which was 0.01. A non-parametric Mann–Whitney test was thereafter applied to compare the means, and the statistical significance was set to \(P < 0.05\).

Results

All the rats subjected to kaolin injection were indeed hydrocephalic as evidenced by ventriculomegaly involving all ventricles and compression of cortical tissue (Fig. 1). The bench experiment (n = 4, values are mean ± SD) showed that the pH (7.35 ± 0.007), pO2 (31.3 ± 2.1 kPa) and pCO2 (5.17 ± 0.43 kPa) were all at normal values throughout the glucose infusion. Glucose concentration...
rose from 5.8 ± 1.0 mmol/l at the start of the infusion to
36.5 ± 2.6 mmol/l at the end of the infusion, i.e. after
80 min. The concentration of plasma lactate was measured
to be 1.00 ± 0.04 mmol/l prior to the infusion and rose to
3.31 ± 0.51 mmol/l during the infusion.

The incorporation of 13C label into [4-13C]glutamate and
[3-13C] glutamate in the brain during glucose infusion is
shown in Fig. 2. As seen from the figure this incorporation
was lower in the hydrocephalus group as compared with the
control group. These time courses were fitted with a one-
compartment model to determine the rate constant
describing the neuronal TCA cycle and the exchange rate
between α-ketoglutarate and glutamate, which are shown in
Table 1. The dilution flux to account for loss of 13C-labelled
glutamine through exchange with unlabelled glutamine was
0.49 ± 0.02 μmoles/gram/minute in the control group, but
could not be determined in the hydrocephalus animals since
the standard deviation of the mean was larger than the mean
value itself. The rate constant describing the dilution of
labelled 13C lactate through exchange with unlabelled lac-
tate hit lower limit.

Oedema was assessed by comparing wet weight tissue
sample before extraction and the freeze dried pellet after
extraction and the ratio between them was 5.19 ± 0.15 for
controls and 5.61 ± 0.43 for the hydrocephalus group
(P = 0.075). Thus, there might be a slight accumulation of
water in the tissue of the animals with hydrocephalus.

Table 2 gives the concentration of metabolites that were
measured in the cortical tissue samples at the end of the experiment corrected for wet weight. The contents of glutamate, taurine, alanine and N-acetyl aspartate (NAA)
were decreased in the brains of the animals with hydro-
cephalus compared with the control group when corrected
for wet weight.

The content of 13C labelled metabolites and the %−13C
enrichment (for calculation see materials and methods)
were very similar in most metabolites except for glutamine,
where both percent enrichment (28 ± 5% for controls and
22 ± 4% for hydrocephalus rats, P = 0.05) and amount of
label (1.65 ± 0.28 μmol/gram for controls and 1.17 ±
0.32 for hydrocephalus rats P = 0.03) were decreased in
the C-2 position.

Discussion

Several studies have used 31P, 1H and ex vivo 13C MRS to
study hydrocephalus and they all provide evidence for both
a disturbance of cerebral energy metabolism and neuronal
The level of significance was set at $P < 0.05$.

**Table 1** Values of the rate of the neuronal TCA cycle and the exchange rate between α-ketoglutarate and glutamate derived from the fit of the experimental data with the metabolic model

|                          | Control ($n = 3$) | Hydrocephalus ($n = 3$) | $P$-value |
|--------------------------|-------------------|-------------------------|-----------|
| Rate of neuronal TCA cycle | 1.3 ± 0.2         | 0.8 ± 0.4               | 0.275     |
| Exchange rate between α-ketoglutarate and glutamate | 4.1 ± 2.5         | 2.7 ± 2.6               | 0.513     |

All results are expressed as mean ± standard deviation (μmoles/gram/minute) of 5 control animals and 5 rats with induced hydrocephalus. Statistical differences between the two groups were analyzed using non-parametric Mann–Whitney test.

$* =$ The level of significance was set at $P < 0.05$.

**Table 2** The amount of metabolites in the brain tissue of hydrocephalus animals and controls

| Metabolite          | Control ($n = 5$) | Hydrocephalus ($n = 5$) | $P$-value |
|---------------------|-------------------|-------------------------|-----------|
| Glutathione         | 1.12 ± 0.16       | 1.02 ± 0.20             | 0.89      |
| Aspartate           | 1.93 ± 0.20       | 1.69 ± 0.17             | 0.11      |
| Glutamate           | 9.20 ± 0.50       | 7.86 ± 0.97*            | 0.03      |
| Glutamine           | 5.90 ± 0.61       | 5.5 ± 0.51              | 0.15      |
| GABA                | 0.96 ± 0.18       | 0.88 ± 0.04             | 0.33      |
| Taurine             | 5.29 ± 0.63       | 4.54 ± 0.33*            | 0.04      |
| Alanine             | 0.93 ± 0.07       | 0.69 ± 0.15*            | 0.02      |
| NAA                 | 11.73 ± 1.03      | 9.41 ± 1.76*            | 0.03      |
| Myo-inositol        | 4.13 ± 0.55       | 4.12 ± 1.29             | 0.99      |
| Lactate             | 4.58 ± 1.46       | 3.73 ± 1.80             | 0.43      |

All results are expressed as mean ± standard deviation of 5 control animals and 5 rats with hydrocephalus. All concentrations were corrected for wet weight (μmol/g tissue). Statistical differences between the two groups were analyzed using unpaired two-tailed Student’s $t$ test.

$* =$ The level of significance was set at $P < 0.05$.

The present study provides important translational evidence for an understanding of long-term sequels in chronic hydrocephalus. It offers evidence to the fact that hydrocephalus causes subtle but significant alterations in neuronal and myelin dysfunction [8–10]. The current study is the first one in which cerebral metabolism has been investigated using in vivo $^{13}$C MRS in a rodent model of hydrocephalus. This allows the assessment of the TCA cycle rate based on measurements of $^{13}$C labelling time courses in individual animals. Thus, the number of animals used is reduced in contrast to metabolic rate determination using ex vivo $^{13}$C MRS. Despite the poorer resolution in spectra obtained using in vivo MRS, uncertainties in the measurements related to post mortem changes and possible errors in the extraction procedures are avoided.

The rate of the neuronal TCA cycle was not significantly different in the rats with hydrocephalus compared to the control group in the present study. This is in line with results obtained in an ex vivo $^{13}$C MRS study, where no differences were found in $^{13}$C incorporation in glutamate, glutamine, GABA and aspartate [11]. The fact that there were no significant differences in the rate of the neuronal TCA cycle and the exchange rate between α-ketoglutarate and glutamate between the groups indicates that the rats with hydrocephalus were indeed able to maintain normal TCA cycle metabolism. However, due to the small number of animals in each group, only large differences in metabolism will be detected. Also glycolysis appears to be unchanged, since lactate levels were similar in the two groups, in agreement with a previous study [3].

Subtle alterations in neuronal metabolism were detected in rats with hydrocephalus compared with controls in the present study. NAA is a neuronal marker [12], and most glutamate is located in neurons [13]. Concentrations of both these metabolite were decreased in cortex of rats with hydrocephalus compared to those in control rats, pointing to decreased neuronal function in the early stage (acute phase) of hydrocephalus. A similar decrease in glutamate level in rats 2 weeks after hydrocephalus induction was observed by Kondziella et al. 2009 [3] using in vivo $^1$H MRS. However, Kondziella et al. 2002 [14] analyzed metabolites in the entire cerebrum at 2 weeks after hydrocephalus induction and found no such difference. Thus, it can be hypothesized that the largest changes in glutamate concentration take place in the cortex, as both the most recent studies analyzed cortical tissue and not the entire brain. While the NAA level was decreased in the present study, Kondziella et al. 2003 and 2009 [3, 11] reported unchanged levels 2 weeks after kaolin induced hydrocephalus. A similar discrepancy was obtained with alanine, which was decreased in the present study, but not in the aforementioned publications. This might point to slight variations in the induction and possibly severity of the kaolin model of hydrocephalus. Indeed results from $^1$H MRS of brain extracts from Texas rats with hydrocephalus, a genetic model of neonatal hydrocephalus due to aqueduct stenosis with onset in late gestation, revealed a decrease in many metabolites (about 50% reduction in glutamine, glutamate, aspartate, creatinine, NAA, taurine and alanine) compared to controls [15–17], pointing to marked differences on metabolites in different hydrocephalus models.

The present study provides important translational evidence for an understanding of long-term sequels in chronic hydrocephalus. It offers evidence to the fact that hydrocephalus causes subtle but significant alterations in neuronal...
metabolism already early in the course of the disease. These sub-lethal changes, however, if maintained and if ongoing might explain the delayed and programmed neuronal damage as seen in chronic hydrocephalus.

Furthermore, in the present study there is indication of reduction also in astrocyte metabolism, since there was a decrease in labelling and % $^{13}$C enrichment in the C-2 position of glutamine. This position is labelled by repeated turns in the TCA cycle with the same intensity as C-3 and by pyruvate carboxylase, an astrocyte specific enzyme [18, 19]. Since only C-2 was affected and not C-3, the pyruvate carboxylase activity appeared to be slightly decreased in the hydrocephalus group. This is in line with a decrease in astrocyte metabolism observed at 4 weeks after hydrocephalus induction using ex vivo MRS [11]. However, at 6 weeks (chronic hydrocephalus) astrocyte metabolism had returned to controls levels [11].

Ventriculomegaly and brain tissue compression was confirmed with MRI of rats with hydrocephalus which was in line with previous results [3, 9]. Considerable oedema has been detected 4 weeks after kaoline injection [14]. However, at 2 weeks after kaoline injection only a tendency towards elevated tissue water content was seen in the current study, but this did not reach statistical significance. An additional indication of disturbed tissue water content was the finding of altered levels of the osmolyte taurine. However, the amount of taurine was significantly decreased in the hydrocephalus group compared with controls, similar to the findings in Kondziella et al. 2002 [14]. Altered taurine levels can be used to control osmolyte balance in the event of changes in water content.

In conclusion, the present results show that in acute experimental hydrocephalus there is slightly reduced neuronal and astrocytic metabolism, but still the hydrocephalic brain is able to maintain its overall metabolism as measured by the rate of the neuronal TCA cycle. This supports the hypothesis that in hydrocephalus disturbance of CSF dynamics is not the only pathophysiologic factor but that metabolic impairment is also of significance [20]. Sublethal deterioration of brain function in the acute course of hydrocephalus has been shown by experimental and clinical studies of blood flow [21] and the present study adds metabolic evidence to it. Possibly, modern metabolic spectroscopy might be used in the future as a diagnostic tool in human hydrocephalus and assist decision making on treatment.

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References

1. Dixon WE, Heller H (1932) Experimentelle Hypertonie durch Erhöhung des intrakraniellen Druckes. Arch Exp Pathol Pharmacol 166:265–275
2. Hochwald GM, Nakamura S, Camins MB (1981) The rat in experimental obstructive hydrocephalus. Z Kinderchir 34:403–410
3. Kondziella D, Eyzolfsson EM, Saether O et al (2009) Gray matter metabolism in acute and chronic hydrocephalus. Neuroscience 159:570–577
4. Gruetter R (1993) Automatic, localized in vivo adjustment of all first- and second-order shim coils. Magn Reson Med 29:804–811
5. Geddes JW, Wood JD (1984) Changes in the amino acid content of nerve endings (synaptosomes) induced by drugs that alter the metabolism of glutamate and gamma-aminobutyric acid. J Neurochem 42:16–24
6. Henry PG, Criego AB, Kumar A et al (2010) Measurement of cerebral oxidative glucose consumption in patients with type 1 diabetes mellitus and hypoglycemia unawareness using $^{13}$C nuclear magnetic resonance spectroscopy. Metabolism 59:100–106
7. Gruetter R, Seaquist ER, Ugurbil K (2001) A mathematical model of compartmentalized neurotransmitter metabolism in the human brain. Am J Physiol Endocrinol Metab 281:E100–E112
8. Del Bigio MR (1993) Neuropathological changes caused by hydrocephalus. Acta Neuropathol 85:573–585
9. McAllister JP 2nd, Chovan P (1998) Neonatal hydrocephalus. Mechanisms and consequences. Neurosurg Clin N Am 9:73–93
10. Braun KP, van Eijjsden P, Vandertop WP et al (1999) Cerebral metabolism in experimental hydrocephalus: an in vivo 1H and 31P magnetic resonance spectroscopy study. J Neurosurg 91:660–668
11. Kondziella D, Qu H, Ludemann W et al (2003) Astrocyte metabolism is disturbed in the early development of experimental hydrocephalus. J Neurochem 85:274–281
12. Baslow MH (2003) N-acetylaspartate in the vertebrate brain: metabolism and function. Neurochem Res 28:941–953
13. Ottersen OP, Storm-Mathisen J (1985) Different neuronal localization of aspartate-like and glutamate-like immunoreactivities in the hippocampus of rat, guinea-pig and Senegalese baboon (Papio papio), with a note on the distribution of gamma-aminobutyrate. Neuroscience 16:589–606
14. Kondziella D, Ludemann W, Brinker T et al (2002) Alterations in brain metabolism, CNS morphology and CSF dynamics in adult rats with kaolin-induced hydrocephalus. Brain Res 927:35–41
15. Harris NG, Jones HC, Williams SC (1992) MR imaging for measurements of ventricles and cerebral cortex in postnatal rats (H-Tx strain) with progressive inherited hydrocephalus. Exp Neurol 118:1–6
16. Harris NG, Plant HD, Briggs RW et al (1996) Metabolic changes in the cerebral cortex of treated and untreated infant hydrocephalic rats studied using in vitro 31P-NMR spectroscopy. J Neurochem 67:2030–2038
17. Jones HC, Harris NG, Rocca JR et al (1997) Progressive changes in cortical metabolites at three stages of infantile hydrocephalus studied by in vitro NMR spectroscopy. J Neurotrauma 14:587–602
18. Shank RP, Bennett GS, Freytag SO et al (1985) Pyruvate carboxylase: an astrocyte-specific enzyme implicated in the
replenishment of amino acid neurotransmitter pools. Brain Res 329:364–367
19. Yu AC, Drejer J, Hertz L et al (1983) Pyruvate carboxylase activity in primary cultures of astrocytes and neurons. J Neurochem 41:1484–1487

20. Kondziella D, Sonnewald U, Tullberg M et al (2008) Brain metabolism in adult chronic hydrocephalus. J Neurochem 106:1515–1524
21. Klinge PM, Samii A, Muhlendyck A et al (2003) Cerebral hypoperfusion and delayed hippocampal response after induction of adult kaolin hydrocephalus. Stroke 34:193–199