Neurochem Res
DOI 10.1007/s11064-014-1357-4

ORIGINAL PAPER

# Acute Hyperammonemia and Systemic Inflammation is Associated with Increased Extracellular Brain Adenosine in Rats: A Biosensor Study

**Peter Nissen Bjerring · Nicholas Dale · Fin Stolze Larsen**

Received: 30 April 2014 / Revised: 22 May 2014 / Accepted: 3 June 2014


**Abstract** Acute liver failure (ALF) can lead to brain edema, cerebral hyperperfusion and intracranial hypertension. These complications are thought to be mediated by hyperammonemia and inflammation leading to altered brain metabolism. As increased levels of adenosine degradation products have been found in brain tissue of patients with ALF we investigated whether hyperammonemia could induce adenosine release in brain tissue. Since adenosine is a potent vasodilator and modulator of cerebral metabolism we furthermore studied the effect of adenosine receptor ligands on intracranial pressure (ICP) and cerebral blood flow (CBF). We measured the adenosine concentration with biosensors in rat brain slices exposed to ammonia and in a rat model with hyperammonemia and systemic inflammation. Exposure to ammonia in concentrations from 0.15–10 mM led to increases in the cortical adenosine concentration up to 18 μM in brain slices. In vivo recordings showed a tendency towards increased adenosine levels in rats with hyperammonemia and systemic inflammation compared to a control group ($3.7 \pm 0.7$ vs. $0.8 \pm 0.2$ μM, $P = 0.06$). This was associated with a significant increase in ICP and CBF. Intervention with the non-selective adenosine receptor antagonist theophyllamine, the $A_{2A}$ receptor antagonist ZM241385, or the $A_1$ receptor agonist N6-Cyclopentyladenosine did not reduce ICP or CBF. In conclusion, our results show that the adenosine concentration in cortex increases during exposure to ammonia, and is associated with a rise in intracranial pressure and cerebral perfusion. However adenosine receptor antagonism/agonism did not reduce the ICP or CBF which indicates that adenosine may not be of direct importance for these cerebral complications in ALF.

**Keywords** Ammonia · Intracranial hypertension · Inflammation · Adenosine · Cerebral blood flow

## Introduction

Acute liver failure (ALF) is associated with hepatic encephalopathy, impaired autoregulation of cerebral blood flow (CBF), cerebral edema and intracranial hypertension [1]. The pathophysiology of these complications is believed to involve both systemic and cerebral inflammation as well as persistent hyperammonemia leading to altered cerebral metabolism of oxygen, glucose and amino acids [2]. A clinical study of brain metabolism using microdialysis technique in patients with ALF has shown that the cerebral level of hypoxanthine is increased [3]. It can be speculated that the increase in the concentration of this adenine nucleotide derivative is related to increased activity in cerebral purinergic signaling, e.g. extracellular release of adenosine in response to changes in the metabolic conditions of the brain. Adenosine is a well characterized and potent vasodilator that is also involved in cerebral autoregulation of blood flow [4], and could be a central mediator in the pathogenesis of brain edema through induction of inappropriate cerebral hyperperfusion and interference with normal autoregulation.

Based on this idea we wanted to study the extracellular level of adenosine in both rat brain tissue exposed to

P. N. Bjerring (✉) · F. S. Larsen
Department of Hepatology, Rigshospitalet, 2100 Copenhagen, Denmark
e-mail: peterbjerring@gmail.com

N. Dale
School of Life Sciences, University of Warwick, Coventry, UK

Published online: 13 June 2014

ammonia in vitro and in an in vivo animal model with hyperammonemia and systemic inflammation. As adenosine has a very short half-life and is rather unstable in vivo [5], we used real-time detection of adenosine with microelectrode biosensors instead of the more conventional use of microdialysis. Thereby we achieved a better signal-to-noise ratio and temporal resolution and also minimized tissue trauma. We hypothesized that the extracellular concentration of adenosine in brain cortex would increase over time during hyperammonemic conditions and furthermore that intervention with adenosine receptor ligands would reduce the intracranial pressure (ICP) and CBF. In order investigate this, we studied the effect of the non-selective adenosine receptor antagonist theophylline, the antagonist ZM241385 selective for the $A_{2A}$ receptor involved in regulation of CBF [6], and the $A_1$ receptor agonist N6-Cyclopentyladenosine (N-CPA) with neuroprotective features demonstrated in various ischemic models [7]. Since systemic $A_1$ receptor agonism is associated with severe bradycardia and hypotension we pretreated these animals with the $A_1$ receptor antagonist 8-(p-Sulfophenyl) theophylline which does not cross the blood–brain-barrier [8].

## Methods

### Facilities

The experiments were performed in School of Life Sciences, University of Warwick, Coventry, UK and at the animal facilities associated with the Laboratory of Hepatology, Rigshospitalet, Copenhagen, Denmark.

### Adenosine Receptor Ligands

Theophylline was purchased as theophylline-ethylenediamine-hydrate (SAD, Amgros, Copenhagen, Denmark) dissolved in sterile water at a concentration of 22 mg/ml. 25 mg of ZM241385 (Tocris Bioscience, Bristol, UK) was dissolved in 1 ml of polyethylene glycol-400 and 1 M NaOH (50:50 vol%) and diluted in isotonic saline at 50 °C to a concentration of 4 mg/ml. 8-SPT (Sigma-Aldrich Denmark, Brøndby, Denmark) was dissolved in isotonic saline at a concentration of 10 mg/ml. N-CPA (Tocris Biomedical) was dissolved in isotonic saline at a concentration of 1 mg/ml.

### Experiment A: Biosensor Study of Cortical Adenosine—In Vitro Model

Brain slices including frontal cortex were obtained from 18 to 30-day-old, male Sprague–Dawley rats. In accordance with the UK Animals (Scientific Procedures) Act 1986, the rats were humanely sacrificed via cervical dislocation. The brain was the rapidly removed and placed in <−4 °C artificial cerebrospinal fluid (aCSF; composed as an aqueous solution of 124 mM NaCl, 3 mM KCl, 2 mM $CaCl_2$, 26 mM $NaHCO_3$, 1.2 $NaH_2PO_4$, 1 mM $MgSO_4$, 10 mM glucose and saturated with 95 %:5 % $O_2$:$CO_2$ with a pH of 7.4). Coronals slices of 300 µm thickness were cut on a Microm HM 650 V microslicer (Carl Zeiss, Welwyn Garden City, UK) and kept in aCSF at room temperature for at least 1 h prior to the experiments. Individual slices were placed in a chamber on a nylon net. The chamber was perfused with recycling aCSF at a temperature of 32–33 °C with a flow-rate of 5–6 ml/min. Microelectrode biosensors (Sarissa Biomedical Ltd, Coventry, UK), one sensitive to adenosine and inosine (ADO) and one lacking enzymes (Null) were placed into the middle of the frontal cortex corresponding to layer 5. After an equilibration period of 20–40 min the experiments were started and the aCSF was exchanged with ammonium acetate dissolved in aCSF in concentrations of 0.15, 0.5, 2, 5, or 10 with an exposure time of 60–75 min. The biosensor signals were measured on a potentiostat (Duo-Stat ME200+, Sarissa Biomedical Ltd) and acquired through an A/D-converter (DT3010, Data Translation, Bietigheim, Germany) connected to a PC running customized software. After the experiments the sensors were retracted from the brain slice and calibrated with aCSF containing 10 µM adenosine. The signal difference between the ADO and Null sensor was then used as a measure of the tissue concentration of adenosine and inosine. The difference from baseline to the peak level of adenosine and the time to peak was identified in each slice. If an increase could not be identified the peak difference was set to zero.

### Experiment B: Biosensor Study of Cortical Adenosine—In Vivo Model

Male Wistar rats of 275–400 g (Charles River, Sulzfeld, Germany) were housed in plastic cages with free access to water and rodent chow, and kept at constant room temperature and humidity with a 12/12-h light/dark cycle. After induction of anaesthesia with isoflurane, 0.2–0.3 ml pentobarbital (50 mg/ml) was administered in a tail vein. Every 10 min, the reaction to claw pinching was checked and supplementary pentobarbital given when necessary. Arterial and venous catheters (PE-50) were inserted in femoral vessels to monitor blood pressure and for intravenous (IV) drug administration and blood sampling. The arterial catheters were flushed with 500 IU heparin and one connected to a pressure transducer. The rats were then tracheotomized and mechanically ventilated (Hallowell EMV, E-vet, Haderslev, Denmark) with a respiratory

frequency of 65 breaths/minute and a tidal volume of 5–10 ml with a mixture of atmospheric air and oxygen adjusted to maintain a normal arterial partial pressure of carbon dioxide and avoid hypoxia during the whole experiment. Arterial blood samples were taken every 30 min for analysis of $pO_2$ and $pCO_2$ (ABL 505; Radiometer, Copenhagen, Denmark). The animals were placed in a stereotactic frame and, with the head fixated, a midline scalp incision was made and one small borehole was drilled in the skull. Through the borehole a catheter (PE-10) was placed in cisterna magna to monitor the intracranial pressure (ICP). Continuous measurements of mean arterial pressure (MAP) and ICP were recorded on a computer using the software Perisoft (Perimed, Stockholm, Sweden). During the experiment body temperature was monitored with an intraabdominal thermistor and maintained at 37 °C with a ventral heading pad.

A cranial window measuring approximately 4 × 6 mm was carefully cut with a dental burr and exposed dura mater over the frontal and parietal cortex. A laser Doppler probe (Probe 407, Perimed, Stockholm, Sweden) was placed close to the brain surface and connected to a Laser Doppler System 5000 monitor (Perimed) in order to measure CBF velocity. Then a plastic cylinder was fixated to the bone surrounding the cranial window by the use of dental cement and served the purpose of an in situ perfusion chamber. Microelectrode biosensors (Sarissa Biomedical Ltd) sensitive to adenosine + inosine (ADO) and inosine (INO) were connected to a potentiostat (Duo-Stat ME200+, Sarissa Biomedical Ltd) and polarized in the perfusion chamber containing a recycling Buffer A (pH 7.4, 10 mM NaPi, 100 mM NaCl, 1 mM $MgCl_2$, 2 mM glycerol) at a flow rate of 5 ml/min. After puncture of dura mater the biosensors were inserted into the frontal cortex.

After an initialization period of 10–15 min with stable baseline values of MAP, ICP and CBF the experiment was started (t = 0). Rats were randomized to either an active group receiving lipopolysaccharide (LPS; Escherichia coli 0127 B8, Sigma-Aldrich) and ammonia-infusion (Sigma-Aldrich) or a control group receiving equal volumes of saline. The animals were given LPS in quantities of 1 mg/kg intraperitoneally (IP) and 1 mg/kg IV or saline and subsequently infusion of ammonia (140 μmol/kg/min IV) or saline for 80 min. At the end of the experiment the biosensors were withdrawn from the cortical tissue. Then Buffer A followed by 10 μM adenosine in Buffer A was flushed over the sensors to obtain a calibration curve. The signal difference between the ADO and INO sensors was used for estimation of tissue concentration of adenosine. Prior to sacrificing the animals by decapitation arterial blood was sampled in order to measure the plasma ammonia concentration. Relative CBF was calculated as percentage of the baseline level. Mean values of MAP, ICP, relative CBF and adenosine concentration over 5 min intervals taken every 15 min were compared between groups. Due to the need for biosensor equilibration after insertion into cortex the first valid signal for analysis was obtained at t = 15 min.

### Experiment C: Effect of Adenosine Receptor Ligands

Male Wistar rats (Charles River) underwent the same procedures described above in experiment B excluding the biosensor insertion. When stable baseline values of MAP, ICP and CBF was obtained for 10 min LPS was injected IP + IV and ammonia IV infusion initiated (t = 0) and continued for 120 min. The rats were randomized to receive IP injections of either (1) theophyllamine 9 mg/kg at t = 20 min, (2) ZM241385 5 mg/kg at t = 20 min and 2.5 mg/kg at t = 80 min, (3) 8-SPT 30 mg/kg at t = 10 min and N-CPA 3 mg/kg at t = 20 min, or (4) an equal volume of isotonic saline. The administered doses were chosen based on previous reports of appropriate CNS activity [9–11]. MAP, ICP and the relative change in CBF was compared between groups at t = 120 min.

### Ethics

All procedures involving laboratory animals were conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and under licenses from the respective national authorities (UK: Animals (Scientific Procedures) Act 1986, Schedule 1; DK: license no. 2012-15-2934-00334).

### Statistics

Results are given as mean ± SEM unless otherwise stated. Linear regression was used for dose–response analysis. Test of normality was done by Shapiro–Wilk's test and logarithmic transformation was used in case on non-normality. Comparison of variables over time and between groups was done by analysis of variance including interaction terms if significant. *P* values less than 0.05 were considered significant.

## Results

### Experiment A

19 brain slices were studied. Adenosine levels peaked after 5–75 min (range) of ammonia exposure. An example of a recording is shown in Fig. 1a. The largest increases in adenosine concentration (up to 18 μM) were seen at the highest ammonia levels at 10 mM. We did not observe

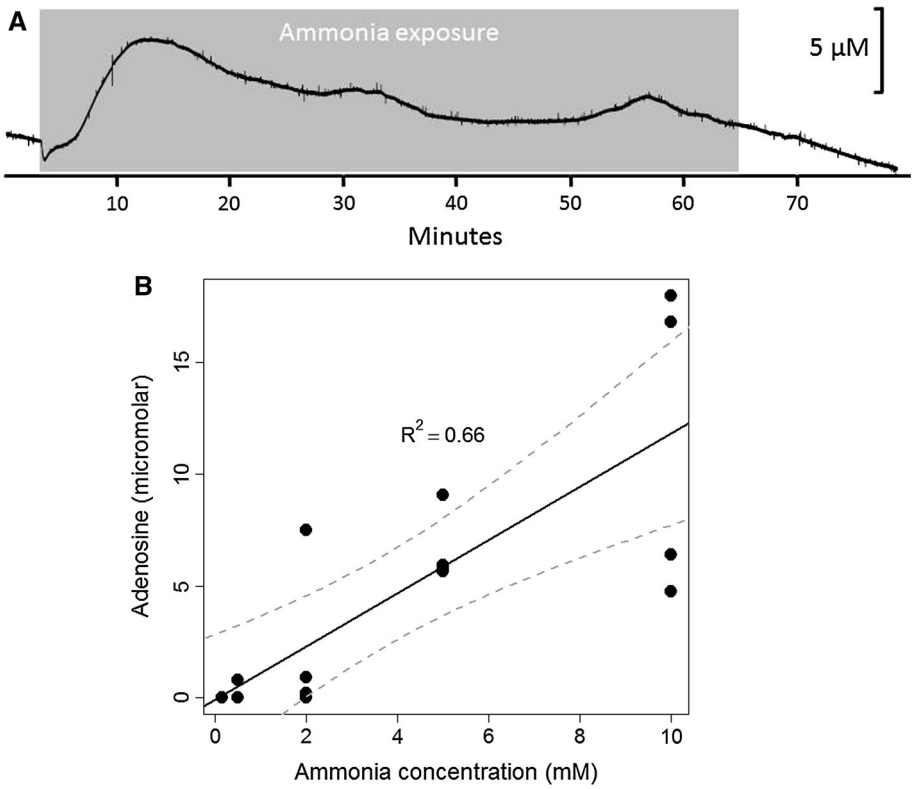


**Fig. 1** **a** Example of a biosensor recording of a rat brain slice exposed to 5 mM ammonia for approximately 60 min. The adenosine level peaks after 10 min and slowly decreases thereafter. **b** Dose–response curve from 19 brain slices exposed to a range of ammonia concentrations from 0.15 to 10 mM. There are overlapping observations in the lower concentrations. A positive linear correlation was found ($P < 0.001$). The *dashed lines* indicate the 95 % confidence interval

increases in adenosine concentration during exposure to 0.15 mM ammonia. We found a linear correlation between the ammonia concentration and the change in adenosine ($P < 0.001$) (Fig. 1b).

Experiment B

MAP was significantly lower in the active group compared to the control group with overall means at 94.0 ± 2.32 versus 105.6 ± 1.7 mmHg (Fig. 2a) ($P < 0.05$ for the main effect of group, without significant interaction with time). ICP increased from 2.2 ± 0.4 to 4.9 ± 0.3 mmHg in the active group and was significantly higher than in the control group (Fig. 2b) [significant interaction between groups and time ($P < 0.001$) and significant simple main effect at t = 75 min ($P < 0.05$)]. CBF increased to 145 ± 9 % compared to 111 ± 7 % in the control group (Fig. 2c) [significant interaction between groups and time ($P < 0.001$) and significant simple main effect at t = 75 min ($P < 0.05$)]. The mean cortical adenosine concentration tended to be higher in the active group compared to the control group at all time points from 15 to 75 min with overall means of 3.7 ± 0.7 versus 0.8 ± 0.2 µM (Fig. 2d). ($P = 0.06$ for the main effect of group, without significant interaction with time. The analysis was done after logarithmic transformation.). There was a tendency towards an increasing adenosine concentration in the active group at the end of the experiment, which however was insignificant. At the end of the experiment, the ammonia plasma level was 2,227 ± 330 µM in the active group compared to 42 ± 4 µM in the control group ($P < 0.0001$).

Experiment C

Treatment with adenosine receptor ligands did not affect MAP, ICP or CBF significantly compared to the control group at t = 120 min (Fig. 3).

## Discussion

Our data show that ammonia exposure results in an increased extracellular concentration of adenosine in rat brain cortex. In the dose–response study of brain slices, marked increases in adenosine levels were seen after exposure to ammonia concentrations above 0.5 mM and adenosine indeed reached physiologically significant concentrations within 1 h. Comparable plasma levels of ammonia can be seen in clinical situations for example ALF [12] or inborn urea cycle disorders [13]. In these patients the hyperammonemic condition can lead to fatal cerebral complications within hours and the degree of hyperammonemia correlates to the prognosis [12, 13].

In this study we also measured the adenosine release in vivo in a rat model of acute hyperammonemia and

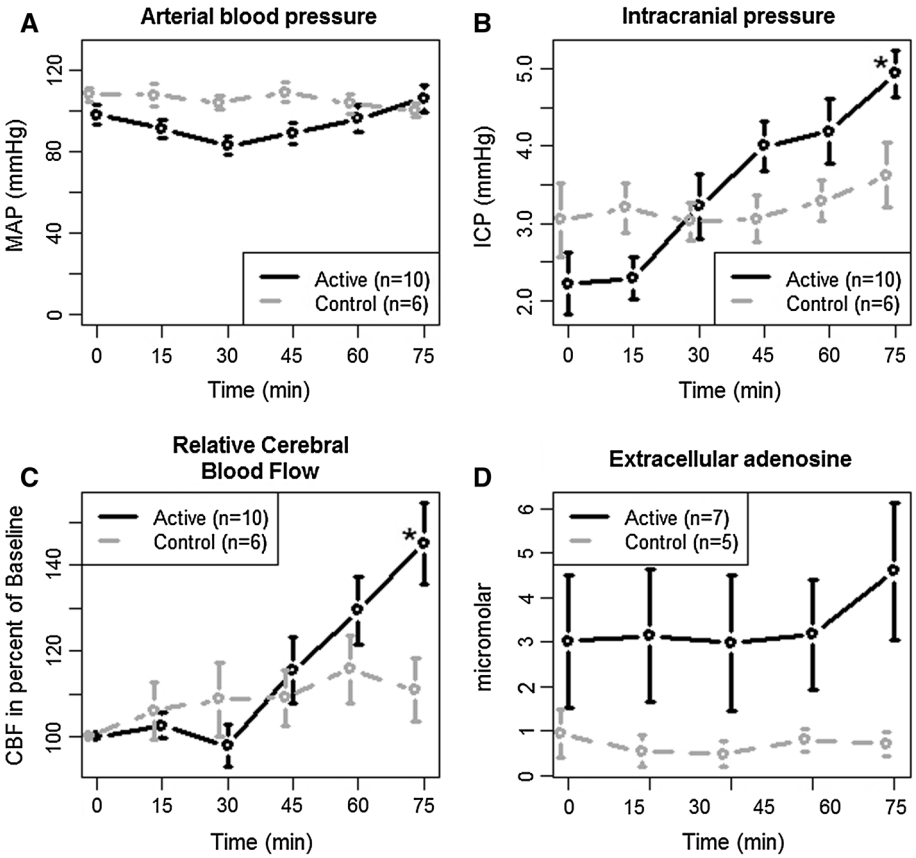


**Fig. 2** **a** The mean arterial pressure (MAP) was significantly lower in the active group (lipopolysaccharide + ammonia) compared to the control group (saline + saline). **b** The intracranial pressure (ICP) increased over time in the active group and was significantly higher than the control group at t = 75 min. *$P < 0.05$. **c** The relative cerebral blood flow (CBF) (baseline = 100 %) over time. A significant higher relative CBF was seen in the active group at t = 75 min. *$P < 0.05$. **d** Extracellular adenosine concentrations measured with biosensors in cerebral cortex. A tendency towards higher concentration in the active group was observed ($P = 0.06$) with no significant effect of time

systemic inflammation, a model that resembles cerebral hemodynamics in patients with ALF. The model builds on the synergistic effect of ammonia and LPS and results in significant rises in both ICP and CBF [14]. We found near significant increased levels of adenosine in the active group and observed a tendency towards further increase in adenosine at t = 75 min. The experiments were limited to a time frame of 75–80 min due to sensitivity loss of the biosensors when used in vivo. Although the ammonia levels reached concentrations of 2 mM, the exposure time might have been too short to induce a significant outcome given the proportions of our study groups. It is noteworthy that there was a tendency towards higher adenosine levels at all time points in the active group, which most likely can be attributed to the immediate systemic inflammatory response induced by LPS. This sepsis-like response was also manifested by the development of mild arterial hypotension within 15 min. Since extracellular adenosine is rapidly degraded to inosine and hypoxanthine in the brain our findings are congruent with the clinical observation of increased hypoxanthine levels in brain microdialysate of ALF patients [3]. An animal study of acute hyperammonemia has found corresponding alterations in the brain purine pool [15], although the model used involved extreme hyperammonemia leading to seizures and death within 15 min. Furthermore brain positron emission tomography of cirrhotic patients has demonstrated reduced brain binding capacity for the adenosine receptor tracer which might due to endogenous adenosine binding [16]. The mechanisms behind the adenosine release we observed were not investigated, but recent data suggest that activation of the $Na^+$–$K^+$ ATPase is coupled to adenosine efflux in brain tissue [17] and indeed such an activation is seen in response to hyperammonemia [18].

In our study we wanted to determine the role of adenosine in the development of cerebral hyperperfusion and intracranial hypertension. In order to investigate this we chose three different pharmacological interventions with relevant CNS bioavailability [9–11]. Based on the assumption that adenosine acts as a mediator of cerebral

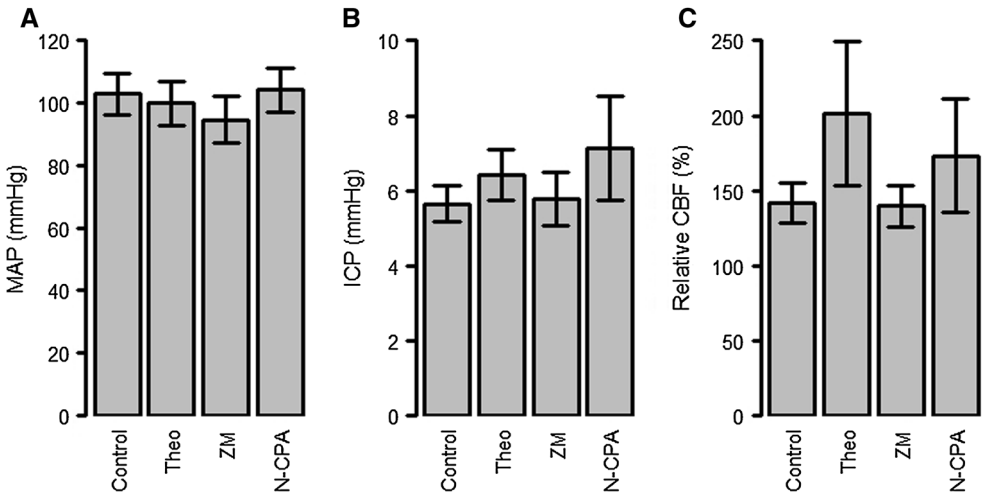


**Fig. 3** **a** Mean arterial pressure (MAP) after 120 min of ammonia infusion. No significant differences were found between the groups. **b** Intracranial pressure after 120 min of ammonia infusion. No significant differences were found between the groups. **c** Relative cerebral blood flow (CBF) (baseline = 100 %) after 120 min of ammonia infusion. No significant differences were found between the groups. Control: lipopolysaccharide + ammonia infusion + saline (n = 6); Theo: lipopolysaccharide + ammonia infusion + theophylline (n = 6); ZM: lipopolysaccharide + ammonia infusion + ZM241385 (n = 6); N-CPA: lipopolysaccharide + ammonia infusion + 8-(p-Sulfophenyl) theophylline + N6-Cyclopentyladenosine (n = 8)

complications through unspecific adenosine receptor activation we studied the effect of the non-selective antagonist theophylline. We did not however find a beneficial effect on ICP or CBF. Given the facts that ALF can lead to impaired cerebral autoregulation [19] and harmful hyperemia [20] and that the adenosine receptor subtype $A_{2A}$ is involved in regulation of CBF [10] we found it interesting to investigate the effect of selective antagonism. However, the antagonist ZM241385 did not attenuate the increased CBF or reduce ICP in our model. Adenosine receptor subtype $A_1$ agonism has been associated with neuroprotection in several diseases such as such as cerebral infarction and neurodegenerative disorders [21]. We evaluated the effect of the agonist N-CPA preceded by systemic $A_1$ receptor antagonism in order to avoid cardiac side effects. Although a normal MAP was preserved we were not able to influence ICP or CBF in a favorable way. Taken together, none of the adenosine receptor ligand interventions had the ability to mitigate the progression of cerebral complications in our model. This can be attributed to one or more of the following reasons: (1) the observed adenosine release is not involved in the development of high ICP and CBF; (2) the systemic side effects of the chosen ligands and doses interferes with the neuroprotective features; (3) the endogenous function of adenosine is more complex than hypothesized, i.e. regional differences or non-linear dose–response behavior and therefore simple ligand interventions with systemic administration can result in unpredictable effects; (4) The CNS bioavailability of the chosen ligands was not sufficient for adequate response. The presented data from our study do not allow us to deduce which of these explanations could be more likely to explain our results.

In perspective, the association between ammonia exposure and cerebral adenosine release could be implicated in aspects of the phenotype of hepatic encephalopathy additional to those examined in our study. In the present study we did not investigate the autoregulation of CBF. Even though we did not find indications of adenosine being involved in the cerebral hyperperfusion per se, abnormal adenosine signaling in the neurovascular unit could still play a role in the impaired autoregulation. Adenosine could hypothetically be the responsible mediator of the increased lower limit of autoregulation and increased autoregulatory index seen in rats with loss of liver mass [22]. The role of adenosine in the matching of CBF and brain metabolism involves activation of potassium channels [4]. Therefore our findings could be in relation to the observation that ammonia exposure leads to potassium disturbances in the brain [23], which in turn is an essential pathophysiological event in ammonia neurotoxicity [24]. Additionally, adenosine also plays an important role in sleep homeostasis [25] and a large proportion of patients with liver failure in fact suffer from sleep disturbances [26], this could be due to alterations in adenosine signaling.

In conclusion, our results show that the adenosine concentration in cortical tissue increases during exposure to high ammonia concentrations, and is associated with a rise in intracranial pressure and cerebral perfusion. However, adenosine receptor antagonism/agonism did not reduce ICP or CBF. This suggests that the adenosine release may be of no direct importance for development of these cerebral complications in ALF.

**Conflict of interest** Nicholas Dale is a founder and shareholder in Sarissa Biomedical Ltd.

## References


1. Bjerring PN, Eefsen M, Hansen BA, Larsen FS (2009) The brain in acute liver failure. A tortuous path from hyperammonemia to cerebral edema. Metab Brain Dis 24(1):5–14
2. Bjerring PN, Larsen FS (2013) Changes in cerebral oxidative metabolism in patients with acute liver failure. Metab Brain Dis 28(2):179–182
3. Bjerring PN, Hauerberg J, Jorgensen L, Frederiksen HJ, Tofteng F, Hansen BA et al (2010) Brain hypoxanthine concentration correlates to lactate/pyruvate ratio but not intracranial pressure in patients with acute liver failure. J Hepatol 53(6):1054–1058
4. Phillis JW (2004) Adenosine and adenine nucleotides as regulators of cerebral blood flow: roles of acidosis, cell swelling, and KATP channels. Crit Rev Neurobiol 16(4):237–270
5. Klinger M, Freissmuth M, Nanoff C (2002) Adenosine receptors: g protein-mediated signalling and the role of accessory proteins. Cell Signal 14(2):99–108
6. Shin HK, Park SN, Hong KW (2000) Implication of adenosine A(2A) receptors in hypotension-induced vasodilation and cerebral blood flow autoregulation in rat pial arteries. Life Sci 67(12):1435–1445
7. Schubert P, Ogata T, Marchini C, Ferroni S, Rudolphi K (1997) Protective mechanisms of adenosine in neurons and glial cells. Ann N Y Acad Sci 15(825):1–10
8. Baumgold J, Nikodijevic O, Jacobson KA (1992) Penetration of adenosine antagonists into mouse brain as determined by ex vivo binding. Biochem Pharmacol 43(4):889–894
9. Morii S, Ngai AC, Ko KR, Winn HR (1987) Role of adenosine in regulation of cerebral blood flow: effects of theophylline during normoxia and hypoxia. Am J Physiol 253(1 Pt 2):H165–H175
10. Kusano Y, Echeverry G, Miekisiak G, Kulik TB, Aronhime SN, Chen JF, et al. (2009) Role of adenosine A2 receptors in regulation of cerebral blood flow during induced hypotension. J Cereb Blood Flow Metab
11. Okamura N, Hashimoto K, Shimizu E, Kumakiri C, Komatsu N, Iyo M (2004) Adenosine A1 receptor agonists block the neuropathological changes in rat retrosplenial cortex after administration of the NMDA receptor antagonist dizocilpine. Neuropsychopharmacology 29(3):544–550
12. Clemmesen JO, Larsen FS, Kondrup J, Hansen BA, Ott P (1999) Cerebral herniation in patients with acute liver failure is correlated with arterial ammonia concentration. Hepatology 29(3):648–653
13. Uchino T, Endo F, Matsuda I (1998) Neurodevelopmental outcome of long-term therapy of urea cycle disorders in Japan. J Inherit Metab Dis 21(Suppl 1):151–159
14. Pedersen HR, Ring-Larsen H, Olsen NV, Larsen FS (2007) Hyperammonemia acts synergistically with lipopolysaccharide in inducing changes in cerebral hemodynamics in rats anaesthetised with pentobarbital. J Hepatol 47(2):245–252
15. Kaminsky Y, Kosenko E (2009) Brain purine metabolism and xanthine dehydrogenase/oxidase conversion in hyperammonemia are under control of NMDA receptors and nitric oxide. Brain Res 6(1294):193–201
16. Boy C, Meyer PT, Kircheis G, Holschbach MH, Herzog H, Elmenhorst D et al (2008) Cerebral A1 adenosine receptors (A1AR) in liver cirrhosis. Eur J Nucl Med Mol Imaging 35(3):589–597
17. Sims RE, Dale N (2014) Activity-dependent adenosine release may be linked to activation of Na(+)-K(+) ATPase: an in vitro rat study. PLoS One 9(1):e87481
18. Kosenko E, Kaminsky Y, Grau E, Minana MD, Marcaida G, Grisolia S et al (1994) Brain Atp depletion induced by acute ammonia intoxication in rats is mediated by activation of the Nmda receptor and Na+, K+-Atpase. J Neurochem 63(6): 2172–2178
19. Strauss G, Hansen BA, Kirkegaard P, Rasmussen A, Hjortrup A, Larsen FS (1997) Liver function, cerebral blood flow autoregulation, and hepatic encephalopathy in fulminant hepatic failure. Hepatology 25(4):837–839
20. Aggarwal S, Kramer D, Yonas H, Obrist W, Kang Y, Martin M et al (1994) Cerebral hemodynamic and metabolic changes in fulminant hepatic failure: a retrospective study. Hepatology 19(1):80–87
21. Rivera-Oliver M, Diaz-Rios M (2014) Using caffeine and other adenosine receptor antagonists and agonists as therapeutic tools against neurodegenerative diseases: a review. Life Sci 101(1–2): 1–9
22. Dethloff TJ, Knudsen GM, Larsen FS (2008) Cerebral blood flow autoregulation in experimental liver failure. J Cereb Blood Flow Metab 28(5):916–926
23. Stephan J, Haack N, Kafitz KW, Durry S, Koch D, Hochstrate P et al (2012) Kir4.1 channels mediate a depolarization of hippocampal astrocytes under hyperammonemic conditions in situ. Glia 60(6):965–978
24. Rangroo TV, Thrane AS, Wang F, Cotrina ML, Smith NA, Chen M et al (2013) Ammonia triggers neuronal disinhibition and seizures by impairing astrocyte potassium buffering. Nat Med 12:1643–1648
25. Basheer R, Porkka-Heiskanen T, Strecker RE, Thakkar MM, McCarley RW (2000) Adenosine as a biological signal mediating sleepiness following prolonged wakefulness. Biol Signals Recept 9(6):319–327
26. Montagnese S, De PC, De RM, Corrias M, Turco M, Merkel C et al (2014) Sleep-wake abnormalities in patients with cirrhosis. Hepatology 59(2):705–712