Chronic ketamine administration impairs mitochondrial complex I in the rat liver

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Abstract

Aim

Ketamine can induce hepatotoxicity which has been suggested to be dependent on mitochondrial impairment. This study investigated the long-term effects of chronic low-dose ketamine on liver mitochondrial function, oxidative stress parameters, liver histology and glycogen content.

Main methods

Adult rats were administered with saline or ketamine (5 or 10 mg/kg) twice a day for a fourteen-day period in order to mimic chronic treatments. Effects between groups were compared ten days after the treatment had ended. Liver mitochondrial function was monitored in isolated mitochondrial extracts through evaluation of respiration parameters and activity of respiratory complexes, as well as oxidative stress, through lipid peroxidation, protein oxidation and superoxide dismutase activity. The hepatic histology and liver glycogen content were also evaluated.

Key findings

Ketamine groups showed a decreased evolution in body weight gains during the treatment period. Ketamine had no effect either on serum liver enzymes or on the oxidative stress parameters of liver mitochondria.

Ketamine decreased the hepatic glycogen content, inhibited mitochondrial complex I and oxygen consumption when glutamate–malate substrate was used.

Significance

These findings reflect a long-term mitochondrial bioenergetic deterioration induced by ketamine, which may explain the increased susceptibility of some patients to its prolonged or repeated use.

Keywords

Ketamine; Mitochondria; Oxidative stress; Liver; Bioenergetics; Glycogen

Introduction

Ketamine has gained a renewed interest for depression and chronic pain management, since it has an immediate and persistent effect on a high percentage of cases in which the classic therapeutic is not effective (Correll et al., 2004 and Zarate et al., 2006). Moreover, ketamine is being increasingly used as a recreational drug (Morgan et al., 2010). However, hepatotoxic side effects have been frequently described after repeated exposure to it (Correll et al., 2004 and Noppers et al., 2011). Ketamine's exact biochemical mechanism for hepatotoxicity is not fully understood, although mitochondrial dysfunction implications have been suggested as a possible explanation (Noppers et al., 2011). The liver is a very active metabolic organ with a high number of mitochondria and very susceptible to drugs acting on mitochondria, which may interfere with bioenergetic function and redox homeostasis (Boelsterli and Lim, 2007 and Szewczyk and Wojtczak, 2002).

Ketamine is metabolised in the liver by the cytochrome P-450 microsomal system (Chan et al., 2005). In human patients, hepatic injuries have been reported after ketamine anaesthetic infusions (Blunnie et al., 1981 and Kiefer et al., 2008) and in the course of long chronic pain treatments (Correll et al., 2004 and Noppers et al., 2011). In vitro studies suggest that mitochondrial dysfunction has an important role on ketamine liver induced injuries (Chang et al., 2009, Lee et al., 2009 and Markham et al., 1981). When exposed to ketamine, hepatocytes showed an inhibition of oxidative phosphorylation (Chang et al., 2009), increased apoptotic insults (Lee et al., 2009) and reactive oxygen species (ROS) production (Reinke et al., 1998). Contrarily, ketamine also showed hepatoprotective effects which are attributed to its anti-inflammatory properties (Suliburk et al., 2005).

Despite several in vitro studies (Chang et al., 2009, Lee et al., 2009 and Markham et al., 1981), there is still little information on ketamine chronic effects on liver mitochondria and oxidative stress responses when in vivo models are used. Very recently, Kalkan et al. (2013) have referred hepatic pathologic changes with mitochondrial degeneration in rats which had been administered high doses of ketamine for two weeks (Kalkan et al., 2013). In addition, we had previously reported that rats subject to a similar period of chronic

low doses of ketamine showed persistent behavioural alterations and loss of weight gain (Venancio et al., 2011). Thus, this study was designed to look into the long-term effects on the liver mitochondrial bioenergetic function, oxidative stress parameters and liver glycogen content following a chronic low dose ketamine treatment in adult rats.

Material and methods

Animals and drug treatment

All procedures used were approved by the local ethical committee and by the Portuguese Agency for Animal Welfare, General Board of Veterinary Medicine, in compliance with the European Community Council Directive of September 22, 2010 (2010/63/UE). All efforts were made to ensure minimal animal stress and discomfort.

Twenty-one adult male Wistar rats (90–110 days) acquired from Charles River (Barcelona, Spain) were randomised in three groups. Animals were kept under controlled environmental conditions of temperature (20 ± 1 °C), relative air humidity (45–55%), maintained in a 12-h light/dark cycle and housed in groups of two or three per cage with standard rodent food and water supplied ad libitum.

Ketamine (Imalgene1000® Merial, Portugal, 100 mg/ml) was freshly diluted before subcutaneous injection in a volume of 1 ml/kg of body weight. Groups were administered with saline solution (K0), 5 mg/kg ketamine (K5) or 10 mg/kg ketamine (K10). Doses were established in accordance with previous studies for analgesic (Wang et al., 2000) or antidepressant properties in rats (Li et al., 2010). Injections were given twice daily (9:00/21:00 h) for 14 consecutive days to achieve a chronic effect (Garcia et al., 2008). The body weight gains were monitored daily throughout the experimental period. Rats were killed 10 days after the last administration (24th day of experiment) by decapitation and blood samples were obtained. The liver was rapidly removed for mitochondrial isolation, histology analysis and glycogen determination.

Reagents

Substrates, enzymes and standard chemical reagents were of the highest grade commercially available and obtained from Sigma (Sigma-Aldrich, Steinheim, Germany). All solutions were prepared with ultra-pure water purified by a Milli-Q Gradient system (Millipore, Bedford, USA).

Liver enzyme activity measurements

The measurement of the hepatocellular enzymes alanine aminotransferase, aspartate aminotransferase and gamma-glutamyl transferase in the blood stream was used as an index of hepatic injury. Blood samples were stored at 4 °C for 1 h; after clotting, they were centrifuged for 10 min at 2800 *g*. Serum levels of

enzyme activity were measured at 37 °C by standard clinical chemical methods using an automatic analyzer type Architect ci8200 (Abbott Laboratories Ltd., USA).

Liver histology

Liver samples were processed by standard techniques and submitted to histologic examination (light microscopy). Briefly, samples were immersed in 10% buffered formalin and embedded in paraffin wax prior to sectioning. For each sample, three series of three sections (3 μm thick) were cut and stained with haematoxylin and eosin or periodic acid-Schiff (PAS) reagent method to detect glycogen as previous described (Bancroft and Stevens, 2002). Diastase for glycogen digestion was used as control. Morphologic analysis was made by a pathologist who was blinded to the experimental protocol. The hepatic morphologic characteristics and increase of areas with glycogen (PAS +) sensitive to digestion with diastase were assessed. The selection of hepatic areas for glycogen content evaluation in hepatocytes was done according to previously described methods (Stadler et al., 2005). Digital photomicrographs of each section were taken (Nikon 4500 Coolpix, USA). Each sample section was graded as follows for PAS + areas: mild (+) — all areas showing slight PAS +; moderate (++) — a combination of areas with slight PAS + and others with intense PAS + staining; high (+++) — extensive and marked areas with intense PAS + staining.

Glycogen analysis

Liver homogenate was used for glycogen concentration determination using phenol–sulphuric method as described previously (Bennett et al., 2007). This was read at 490 nm, 30 min after incubation (Cary 100 Bio, Varian Analytical Instruments, USA).

Isolation of liver mitochondria

The isolation was performed as described previously (Peixoto et al., 2002). The homogenization medium contained 225 mM mannitol, 75 mM sucrose, 10 mM Hepes (pH 7.4), 2 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA) and 0.1% fatty acid-free bovine serum albumin (BSA). EGTA and BSA were omitted from the final washing medium. The final concentration of mitochondrial protein was determined by the Biuret method, using BSA as standard.

Liver mitochondrial respiratory activity

Oxygen consumption of isolated mitochondria was monitored polarographically with a Clark-type oxygen electrode, using a Hansatech Oxygraph measurement system (Hansatech, Norfolk, UK) at 25 °C. Mitochondria (0.5 mg) were incubated, for 2 min in 1 ml of reaction medium (100 mM sucrose, 100 mM KCl, 2 mM KH_2PO_4, 5 mM Hepes, 10 μM EGTA, pH 7.4). The mitochondria were energised with glutamate–malate (complex I substrate) or succinate (complex II substrate). Oxygen consumption was measured in the absence (state 4) or presence of 100 μM ADP (state 3). Respiratory rates were expressed

in nmol O_2/citrate synthase activity. Respiratory control ratio (RCR = state 3/state 4) was calculated (Vilela et al., 2009).

Liver mitochondrial enzymatic activity assays

Before the enzyme assays, the mitochondrial frozen samples (at − 70 °C) were freeze-thawed three times to disrupt their membranes. All the enzymatic assays were performed on a temperature controlled chamber with stirring and were performed in triplicate in a microplate apparatus (Power Wave XS2, Biotek, USA).

Citrate synthase activity was determined as described previously at 30 °C (Shepherd and Garland, 1969) with slight modifications. The reaction mixture contained 100 mM Tris–HCl buffer (pH 8.0), 5 mM $MgCl_2$, 0.5 mM 5,5-dithio-bis-2-nitrobenzoic acid, 0.2 mM acetyl CoA, 1 mM oxaloacetic acid and broken mitochondria (15–25 μg). The decrease of acetyl CoA absorption was monitored at 412 nm and specific activity, as units of citrate synthase activity/min · mg of protein, was calculated and used to normalise the other mitochondrial functional parameters.

The electron transport chain complexes I, II, III and IV were quantified by spectrophotometric assay as previously described (Kiebish et al., 2008). Complex V (ATPase) activity was determined by monitoring the pH change associated with ATP hydrolysis (Peixoto et al., 2002).

Liver mitochondrial oxidative stress parameters

Lipid peroxidation was determined measuring malondialdehyde equivalents, using the thiobarbituric acid assay, as previously described (Alves et al., 2007). Protein carbonyls were quantified through the spectrophotometric carbonyl assay, as recently described (Hawkins et al., 2009), using 2, 4-dinitrophenylhydrazine. Superoxide dismutase activity was determined as described previously (Paya et al., 1992).

Statistical analysis

Comparisons were made by one-way ANOVA followed, when appropriated, by Tukey's post hoc test. Repeated-measures ANOVA was used for weight gain analysis. Results are expressed as mean ± SEM. The statistical level of significant was considered at $p < 0.05$, using the GraphPad Prism 6.0 (GraphPad Software, San Diego, CA, USA).

Results

Body weight gain

The chronic ketamine administrations induced a decrease in body weight gain during the experimental period $F(2,17) = 32.44$, $p < 0.0001$, (Fig. 1). No mortality was observed between groups; however, one rat

was excluded for displaying aggressive behaviour. The results revealed decreased body weight gain among the K0 and the ketamine groups after the second day of treatment ($p < 0.05$). The weight recovery started on day ten for both ketamine groups. The K5 group showed less accentuated differences and after the twenty-second day no significant differences were observed by comparison with K0 group ($p = 0.79$). Both groups failed to reach the control values. Moreover, except for day two, the K10 group showed a significant reduction in body weight gain compared to the K5 group ($p < 0.05$).

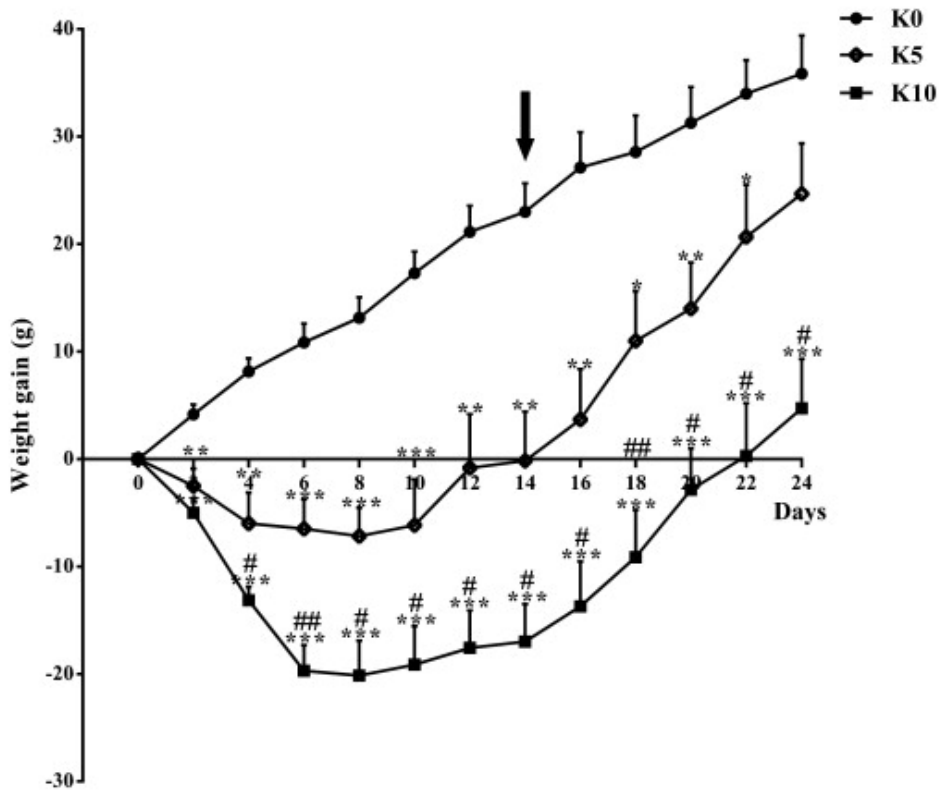

Fig. 1.

Effect of chronic ketamine administration on weight gain throughout the experimental period. The arrow indicates the end of the administration period. Each point represents the mean ± SEM for each group of body weight in a given day. Saline solution (K0, n = 7); 5 mg/kg ketamine (K5, n = 6); 10 mg/kg ketamine (K10, n = 7). Significant differences were marked as $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ for K0; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ between K5 and K10.

Blood evaluation

The activities of biochemical markers of liver damage, such as alanine aminotransferase and aspartate aminotransferase, were not significantly different between groups. The levels of gamma-glutamyl transferase were below the detection limits (< 4 IU/l) in all samples.

Liver histology and glycogen concentration

In all samples, the liver showed normal histological features with no increase in the number of degenerating cells, fibrosis or infiltrated inflammatory cells. However, with recourse to the PAS method (Fig. 2) and by the spectrophotometrical assay using phenol–sulphuric method, we were able to observe that the livers from rats exposed to ketamine displayed lower levels of glycogen concentration than in those of rats from the control group, $F(2,17) = 5.531$, $p = 0.014$, (Fig. 3). Detailed analysis revealed that K10 treatments decreased glycogen content in hepatocytes ($p < 0.05$).

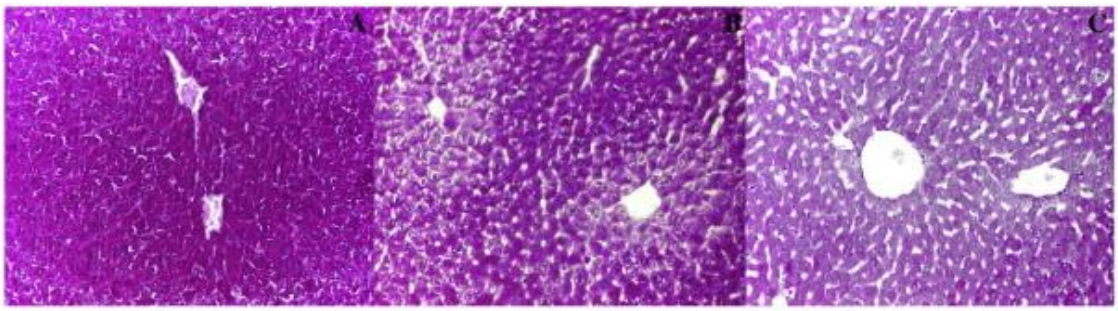

Fig. 2.

Effects of chronic ketamine in liver glycogen content. Histopathologic findings showed a reduction of glycogen in hepatocytes for 5 and 10 mg/kg ketamine compared to control. Representative photomicrographs of liver sections stained with PAS (objective, 20 ×): (A) saline solution; (B) 5 mg/kg ketamine and (C) 10 mg/kg ketamine group.

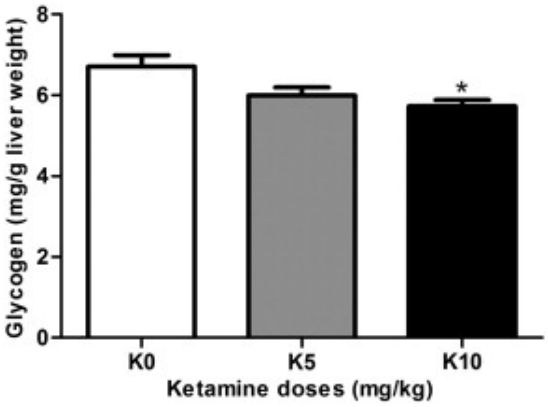

Fig. 3.

Effects of chronic ketamine in liver glycogen content. Results are expressed as means ± SEM (n = 6–7) for each group. K0 (saline solution), K5 and K10 (5 or 10 mg/kg ketamine, respectively). Significant differences for control are marked as *p < 0.05.

Liver mitochondrial functionality

Oxygen consumption with substrates for complex I (glutamate–malate) and II (succinate) was observed. The main effect of treatment in mitochondrial respiration initiated by glutamate–malate was seen in state 3, $F(2,15) = 6.704$, $p = 0.0083$, (Fig. 4A) and state 4, $F(2,15) = 34.84$, $p < 0.0001$, (Fig. 4B). Detailed analysis revealed that, when the respiratory chain was activated by glutamate–malate, both K5 and K10 treatments decreased oxygen consumption in state 3 ($p < 0.05$) and state 4 ($p < 0.0001$). But when succinate was used as substrate, no significant differences were observed in these parameters (Fig. 4D and E).

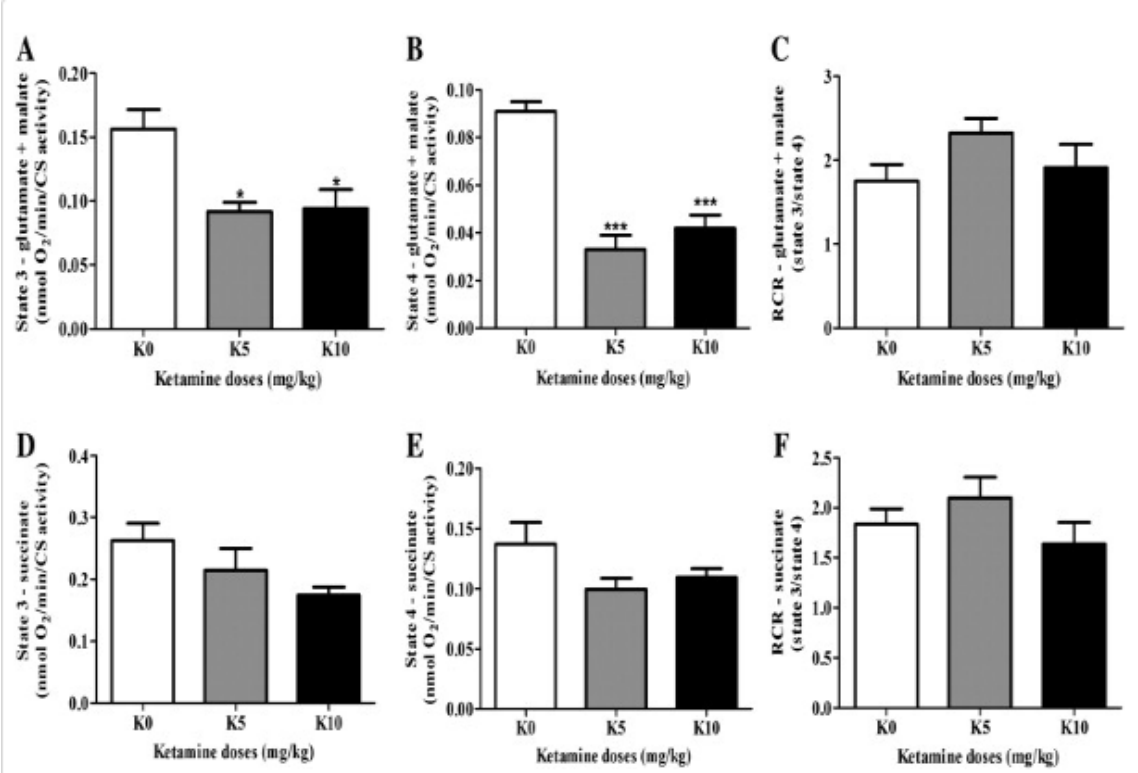

Fig. 4.

Effects of chronic ketamine administration on respiratory parameters evaluated in rat liver mitochondria. (A) State 3 energised with glutamate–malate; (B) state 4 energised with glutamate–malate; (C) RCR energised with glutamate–malate; (D) state 3 energised with succinate; (E) state 4 energised with succinate; (F) RCR energised with succinate. Complex II activity results are expressed as means ± SEM (n = 5–7) for each group. Citrate synthase (CS) activities were used to normalise mitochondrial respiratory activities. RCR — respiratory control ratio. K0, K5 and K10: saline solution, 5 or 10 mg/kg ketamine, respectively. Significant differences for control are marked as *$p < 0.05$ and ***$p < 0.001$.

The respiratory control ratio is a measure of the dependence on the respiratory rate with oxidative phosphorylation. This study showed no significant difference between experimental groups in RCR for both respiratory substrates (Fig. 4C and F).

The activity of complex I was significantly inhibited by ketamine doses $F(2,17) = 14.06$, $p = 0.0002$, (Fig. 5A). Complex I was reduced by 41.1% and 45.6% for K5 ($p < 0.01$) and K10 ($p < 0.001$), respectively, in comparison with control group. The specific enzymatic activities of complexes II, III and IV were not significantly affected by ketamine treatments (Fig. 5B, C and D). The activity of complex V showed a trend to decrease $F(2,17) = 3.133$, $p = 0.0695$, (Fig. 5E), with a reduction of 45.0% and 28.3% in groups K5 and K10, respectively, in comparison with the control group.

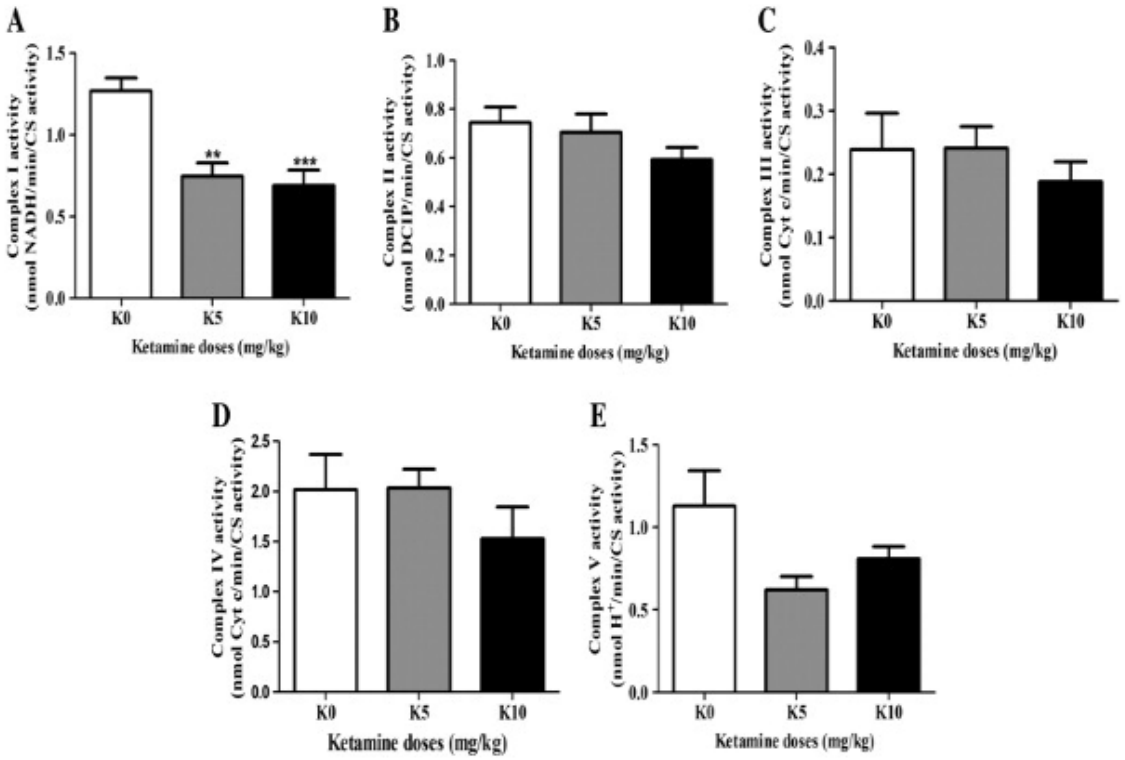

Fig. 5.

Effect of chronic ketamine administration on enzymatic activities of mitochondrial respiratory chain complexes. (A) Complex I activity; (B) complex II activity; (C) complex III activity; (D) complex IV activity; (E) complex V activity. Results are expressed as means ± SEM (n = 6–7) for each group. Citrate synthase (CS) activities were used to normalise enzymatic mitochondrial activities. DCIP — 2,6-dichloroindophenol. K0, K5 and K10: saline solution, 5 or 10 mg/kg ketamine, respectively. Significant differences for control are marked as **p < 0.01 and ***p < 0.001.

Liver mitochondrial oxidative stress

The lipid peroxidation, protein carbonyls and superoxide dismutase activity evaluated in liver mitochondria were not significantly different between groups (data not shown).

Discussion

This work shows that chronic ketamine treatment with a lower dose in rats impairs liver mitochondrial complex I and decreases liver glycogen content as well as body weight gain. Nevertheless, neither other hepatic histological changes nor significant hepatotoxicity effects were observed.

The body weight gain is a parameter usually used as a health indicator in chronic rodent toxicology studies (Rhomberg et al., 2007). Chronic low ketamine doses have induced a significant loss of body weight gain. These results are consistent with other previous observations (Venancio et al., 2011). At the end of the experimental period, only the higher ketamine group showed a significant weight reduction. A possible explanation for the decrease of body weight gain could be related to the fact that ketamine induces nausea

and vomiting with consequent loss of appetite as observed in humans (Cvrcek, 2008). Moreover, nausea and vomiting are associated with liver injury (Verma and Kaplowitz, 2009). However, we did not observe alterations in enzymatic biomarkers for liver injury. Similarly, in humans, after chronic pain management with ketamine, no significant alterations of liver enzymes were shown (Amr, 2010, Cvrcek, 2008 and Sigtermans et al., 2009). This could also be the result of biochemical analysis having been performed ten days after the last ketamine administration; also, as it happened with humans, an increase of liver enzymes was observed but it went back to normal values between ten days and two months after the pain treatment had ended (Kiefer et al., 2008 and Noppers et al., 2011). Another likely explanation for the decrease of body weight gain may be related to the fact that, being an N-methyl-D-aspartate (NMDA) receptor antagonist, ketamine may have brain interference in food intake (Popik et al., 2011 and Wu et al., 2012) and also hepatic metabolism regulation implications (Lam et al., 2010).

This study showed a decrease of hepatic glycogen concentration in the higher ketamine group, which was also corroborated by histological observations. Otherwise, no hepatic histological changes were observed. Reduced glycogen store in hepatocytes is related to an increased susceptibility to hepatotoxicity (Stadler et al., 2005). In mice, chronic ketamine treatment also decreases glycogen in liver, reflecting either a failure of glycogen synthesis or an increase of glycogenolysis (Wong et al., 2012). Other studies also reported that ketamine can promote hepatic bioenergetic deterioration with concomitant reduction of ATP production (Takahashi et al., 1998), gluconeogenesis (Sear and McGivan, 1979) and increased glycogenolysis (Machado et al., 2009) which may be associated with an increase of blood glucose levels (Dawson et al., 2013 and Rodrigues et al., 2006).

Hepatic bioenergetic deterioration reflected by the decrease in glycogen is usually coupled with reduction of ATP production (Gallis et al., 2011). Ketamine decreased the oxygen consumption in state 3 and state 4 when complex I was energised. This may result from the observed inhibition of complex I (NADH ubiquinone oxidoreductase) activity. Similar effects of ketamine in hepatic cell cultures have been recently observed such as time-dependent interruption of microtubular cytoskeleton remodelling, decreased intracellular levels of calcium concentrations and concurrent reduction of ATP production due to down-regulation of the mitochondrial membrane potential and complex I activity (Chang et al., 2009). When succinate was used as substrate, there were neither significant alterations, nor significant change in the activity of other electron transport chain complexes which confirms that the previous reported decrease in oxygen consumption is due to a direct effect on complex I. In line with these findings, it has been reported that ketamine induces mitochondrial complex I impairment on isolated rat liver mitochondria (Markham et al., 1981). However, a normal value of RCR in ketamine groups for the two substrates used also indicates that the structural integrity of mitochondria was not affected. Complex I is the first enzyme of the respiratory chain and plays a central role in oxidative phosphorylation (Efremov et al., 2010). Its inhibition promoted by ketamine and by other anaesthetics may be related to the interference in glucose metabolism (Biebuyck et al., 1972).

Complex I inhibition can exacerbate mitochondrial ROS generation. Nevertheless, due to its antioxidant capacity, mitochondria can rapidly degrade ROS (Brown and Borutaite, 2012). This can account for the absence of lipid peroxidation and protein oxidation in liver mitochondria obtained from rats treated with ketamine. Moreover, the literature concerning ROS production as a result of ketamine anaesthesia is controversial; in fact, several studies show that it decreases (Saricaoglu et al., 2005), or has no effect at all (Alva et al., 2006), while others claim it does, indeed, increase ROS production (Reinke et al., 1998). Furthermore, pre-treatment with ketamine can potentiate hepatotoxicity of other compounds by increasing oxidative stress (Rofael, 2004).

As far as we know, this is the first in vivo low-dose ketamine study to evaluate liver effects, liver mitochondrial function and oxidative stress. A recently published study showed that a two-week high-dose ketamine administration in rat models induced hepatic pathologic changes with mitochondrial degeneration associated with a decreased hepatic expression of calcineurin, a protein phosphatase regulated by calcium (Kalkan et al., 2013). Based on works from Wang et al. (2000) and Garcia et al. (2008), the doses used are within the therapeutic use. The results thus obtained confirmed the critical role of ketamine on mitochondrial complex I impairment and bioenergetic deterioration (Chang et al., 2009 and Markham et al., 1981). Ketamine hepatotoxicity's mechanism is still unclear, but this work has the relevance of showing impairment effects in mitochondrial complex I ten days after the end of chronic ketamine treatment. This effect may be the result of a direct ketamine action in complex I or of a likely decrease in calcium levels, but it also indicates insufficient mitochondrial recovery systems after chronic ketamine treatment. It has been reported that change in the mitochondrial function increases sensitivity to anaesthetics (Morgan et al., 2002). Therefore, on the clinical level, complex I impairment and the decrease in hepatic glycogen content could justify the susceptibility of some patients to prolonged or repeated use of ketamine in humans for chronic pain management (Correll et al., 2004 and Noppers et al., 2011).

Conclusion

In summary, our findings failed to show chronic low dose ketamine effects on ROS production and in enzymatic biomarkers for liver injury. Results showed a decrease of mitochondrial complex I activity and glycogen levels. These liver effects point to the need of precaution in ketamine administration and may explain the increased susceptibility of some patients to its prolonged or repeated use.

References

Alva et al., 2006 N. Alva, J. Palomeque, T. Carbonell Nitric oxide induced by ketamine/xylazine anesthesia maintains hepatic blood flow during hypothermia Nitric Oxide, 15 (1) (2006), pp. 64–69

Alves et al., 2007 E. Alves, T. Summavielle, C.J. Alves, J. Gomes-Da-Silva, J.C. Barata, E. Fernandes, et al. Monoamine oxidase-B mediates ecstasy-induced neurotoxic effects to adolescent rat brain mitochondria J Neurosci, 27 (38) (2007), pp. 10203–10210

Amr, 2010 Y.M. Amr Multi-day low dose ketamine infusion as adjuvant to oral gabapentin in spinal cord injury related chronic pain: a prospective, randomized, double blind trial Pain Physician, 13 (3) (2010), pp. 245–249

Bancroft and Stevens, 2002 J. Bancroft, A. Stevens Theory and practice of histological techniques (5th ed.)Churchill Livingstone, Philadelphia (2002)

Bennett et al., 2007 L.W. Bennett, R.W. Keirs, E.D. Peebles, P.D. Gerard Methodologies of tissue preservation and analysis of the glycogen content of the broiler chick liver Poult Sci, 86 (12) (2007), pp. 2653–2665

Biebuyck et al., 1972 J.F. Biebuyck, P. Lund, H.A. Krebs The effects of halothane (2-bromo-2-chloro-1, 1, 1-trifluoroethane) on glycolysis and biosynthetic processes of the isolated perfused rat liver Biochem J, 128 (3) (1972), pp. 711–720

Blunnie et al., 1981 W.P. Blunnie, M. Zacharias, J.W. Dundee, J.R. Doggart, J. Moore, P.D. Mcilroy Liver enzyme studies with continuous intravenous anaesthesia Anaesthesia, 36 (2) (1981), pp. 152–156

Boelsterli and Lim, 2007 U.A. Boelsterli, P.L. Lim Mitochondrial abnormalities—a link to idiosyncratic drug hepatotoxicity? Toxicol Appl Pharmacol, 220 (1) (2007), pp. 92–107

Brown and Borutaite, 2012 G.C. Brown, V. Borutaite There is no evidence that mitochondria are the main source of reactive oxygen species in mammalian cells Mitochondrion, 12 (1) (2012), pp. 1–4

Chan et al., 2005 W.H. Chan, W.Z. Sun, T.H. Ueng Induction of rat hepatic cytochrome P-450 by ketamine and its toxicological implications J Toxicol Environ Health A, 68 (17–18) (2005), pp. 1581–1597

Chang et al., 2009 H.C. Chang, T.L. Chen, R.M. Chen Cytoskeleton interruption in human hepatoma HepG2 cells induced by ketamine occurs possibly through suppression of calcium mobilization and mitochondrial function Drug Metab Dispos, 37 (1) (2009), pp. 24–31

Correll et al., 2004 G.E. Correll, J. Maleki, E.J. Gracely, J.J. Muir, R.E. Harbut Subanesthetic ketamine infusion therapy: a retrospective analysis of a novel therapeutic approach to complex regional pain syndrome Pain Med, 5 (3) (2004), pp. 263–275

Cvrcek, 2008 P. Cvrcek Side effects of ketamine in the long-term treatment of neuropathic pain Pain Med, 9 (2) (2008), pp. 253–257

Dawson et al., 2013 N. Dawson, B.J. Morris, J.A. Pratt Subanaesthetic ketamine treatment alters prefrontal cortex connectivity with thalamus and ascending subcortical systems Schizophr Bull, 39 (2) (2013), pp. 366–377

Efremov et al., 2010 R.G. Efremov, R. Baradaran, L.A. Sazanov The architecture of respiratory complex I Nature, 465 (7297) (2010), pp. 441–445

Gallis et al., 2011 J.L. Gallis, H. Gin, H. Roumes, M.C. Beauvieux A metabolic link between mitochondrial ATP synthesis and liver glycogen metabolism: NMR study in rats re-fed with butyrate and/or glucose Nutr Metab (Lond), 8 (1) (2011), p. 38

Garcia et al., 2008 L.S. Garcia, C.M. Comim, S.S. Valvassori, G.Z. Reus, A.C. Andreazza, L. Stertz, et al. Chronic administration of ketamine elicits antidepressant-like effects in rats without affecting hippocampal brain-derived neurotrophic factor protein levels Basic Clin Pharmacol Toxicol, 103 (6) (2008), pp. 502–506

Hawkins et al., 2009 C.L. Hawkins, P.E. Morgan, M.J. Davies Quantification of protein modification by oxidants Free Radic Biol Med, 46 (8) (2009), pp. 965–988

Kalkan et al., 2013 Y. Kalkan, Y. Tomak, D. Altuner, L. Tumkaya, H. Bostan, A. Yilmaz, et al. Hepatic effects of ketamine administration for 2 weeks in rats Hum Exp Toxicol (2013), pp. 1–9

Kiebish et al., 2008 M.A. Kiebish, X. Han, H. Cheng, A. Lunceford, C.F. Clarke, H. Moon, et al. Lipidomic analysis and electron transport chain activities in C57BL/6J mouse brain mitochondria J Neurochem, 106 (1) (2008), pp. 299–312

Kiefer et al., 2008 R.T. Kiefer, P. Rohr, A. Ploppa, H.J. Dieterich, J. Grothusen, S. Koffler, et al. Efficacy of ketamine in anesthetic dosage for the treatment of refractory complex regional pain syndrome: an open-label phase II study Pain Med, 9 (8) (2008), pp. 1173–1201

Lam et al., 2010 C.K. Lam, M. Chari, B.B. Su, G.W. Cheung, A. Kokorovic, C.S. Yang, et al. Activation of N-methyl-D-aspartate (NMDA) receptors in the dorsal vagal complex lowers glucose production J Biol Chem, 285 (29) (2010), pp. 21913–21921

Lee et al., 2009 S.T. Lee, T.T. Wu, P.Y. Yu, R.M. Chen Apoptotic insults to human HepG2 cells induced by S-(+)-ketamine occurs through activation of a Bax–mitochondria–caspase protease pathway Br J Anaesth, 102 (1) (2009), pp. 80–89

Li et al., 2010 N. Li, B. Lee, R.J. Liu, M. Banasr, J.M. Dwyer, M. Iwata, et al. mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists Science, 329 (5994) (2010), pp. 959–964

Machado et al., 2009 E.F. Machado, A.C. Normand, L.A. Nunes, R. Brenzikofer, D.V. Macedo Effects of different general anesthetics on serum hemolysis and hepatic and muscular glycogenolysis in rats Braz J Med Biol Res, 42 (11) (2009), pp. 1035–1038

Markham et al., 1981 A. Markham, I. Cameron, S.J. White The effect of ketamine hydrochloride, a non-barbiturate parenteral anaesthetic on oxidative phosphorylation in rat liver mitochondria Biochem Pharmacol, 30 (15) (1981), pp. 2165–2168

Morgan et al., 2002 P.G. Morgan, C.L. Hoppel, M.M. Sedensky Mitochondrial defects and anesthetic sensitivity Anesthesiology, 96 (5) (2002), pp. 1268–1270

Morgan et al., 2010 C.J. Morgan, L. Muetzelfeldt, H.V. Curran Consequences of chronic ketamine self-administration upon neurocognitive function and psychological wellbeing: a 1-year longitudinal study Addiction, 105 (1) (2010), pp. 121–133

Noppers et al., 2011 I.M. Noppers, M. Niesters, L.P. Aarts, M.C. Bauer, A.M. Drewes, A. Dahan, et al. Drug-induced liver injury following a repeated course of ketamine treatment for chronic pain in CRPS type 1 patients: a report of 3 cases Pain, 152 (9) (2011), pp. 2173–2178

Paya et al., 1992 M. Paya, B. Halliwell, J.R. Hoult Interactions of a series of coumarins with reactive oxygen species. Scavenging of superoxide, hypochlorous acid and hydroxyl radicals Biochem Pharmacol, 44 (2) (1992), pp. 205–214

Peixoto et al., 2002F. Peixoto, A.I. Barros, A.M. Silva Interactions of a new 2-styrylchromone with mitochondrial oxidative phosphorylation J Biochem Mol Toxicol, 16 (5) (2002), pp. 220–226

Popik et al., 2011 P. Popik, T. Kos, Y. Zhang, A. Bisaga Memantine reduces consumption of highly palatable food in a rat model of binge eating Amino Acids, 40 (2) (2011), pp. 477–485

Reinke et al., 1998 L.A. Reinke, Y. Kotake, D.R. Moore, A.A. Nanji Free radical formation during ketamine anesthesia in rats: a cautionary note Free Radic Biol Med, 24 (6) (1998), pp. 1002–1006

Rhomberg et al., 2007 L.R. Rhomberg, K. Baetcke, J. Blancato, J. Bus, S. Cohen, R. Conolly, et al. Issues in the design and interpretation of chronic toxicity and carcinogenicity studies in rodents: approaches to dose selection Crit Rev Toxicol, 37 (9) (2007), pp. 729–837

Rodrigues et al., 2006 S.F. Rodrigues, M.A. De Oliveira, J.O. Martins, P. Sannomiya, Tostes R. De Cassia, D. Nigro, et al. Differential effects of chloral hydrate- and ketamine/xylazine-induced anesthesia by the s.c. route Life Sci, 79 (17) (2006), pp. 1630–1637

Rofael, 2004 H.Z. Rofael Effect of ketamine pretreatment on cocaine-mediated hepatotoxicity in rats Toxicol Lett, 152 (3) (2004), pp. 213–222

Saricaoglu et al., 2005 F. Saricaoglu, D. Dal, A.E. Salman, M.N. Doral, K. Kilinc, U. Aypar Ketamine sedation during spinal anesthesia for arthroscopic knee surgery reduced the ischemia–reperfusion injury markers Anesth Analg, 101 (3) (2005), pp. 904–909

Sear and Mcgivan, 1979 J.W. Sear, J.D. Mcgivan Cytotoxicity of i.v. anaesthetic agents on the isolated rat hepatocyte Br J Anaesth, 51 (8) (1979), pp. 733–739

Shepherd and Garland, 1969 D. Shepherd, P.B. Garland The kinetic properties of citrate synthase from rat liver mitochondria Biochem J, 114 (3) (1969), pp. 597–610

Sigtermans et al., 2009 M.J. Sigtermans, J.J. Van Hilten, M.C. Bauer, M.S. Arbous, J. Marinus, E.Y. Sarton, et al. Ketamine produces effective and long-term pain relief in patients with complex regional pain syndrome type 1 Pain, 145 (3) (2009), pp. 304–311

Stadler et al., 2005 M. Stadler, V. Nuyens, L. Seidel, A. Albert, J.G. Boogaerts Effect of nutritional status on oxidative stress in an ex vivo perfused rat liver Anesthesiology, 103 (5) (2005), pp. 978–986

Suliburk et al., 2005 J.W. Suliburk, K.S. Helmer, E.A. Gonzalez, E.K. Robinson, D.W. Mercer Ketamine attenuates liver injury attributed to endotoxemia: role of cyclooxygenase-2 Surgery, 138 (2) (2005), pp. 134–140

Szewczyk and Wojtczak, 2002 A. Szewczyk, L. Wojtczak Mitochondria as a pharmacological target Pharmacol Rev, 54 (1) (2002), pp. 101–127

Takahashi et al., 1998 K. Takahashi, S. Nosaka, S. Morikawa, T. Inubushi Hepatic energy metabolism during ketamine and isoflurane anaesthesia in haemorrhagic shock Br J Anaesth, 80 (6) (1998), pp. 782–787

Venancio et al., 2011 C. Venancio, A. Magalhaes, L. Antunes, T. Summavielle Impaired spatial memory after ketamine administration in chronic low doses Curr Neuropharmacol, 9 (1) (2011), pp. 251–255

Verma and Kaplowitz, 2009 S. Verma, N. Kaplowitz Diagnosis, management and prevention of drug-induced liver injury Gut, 58 (11) (2009), pp. 1555–1564

Vilela et al., 2009 S.M. Vilela, D.J. Santos, L. Felix, J.M. Almeida, L. Antunes, F. Peixoto Are fentanyl and remifentanil safe opioids for rat brain mitochondrial bioenergetics? Mitochondrion, 9 (4) (2009), pp. 247–253

Wang et al., 2000 Y. Wang, C. Huang, Y. Cao, J.S. Han Repeated administration of low dose ketamine for the treatment of monoarthritic pain in the rat Life Sci, 67 (3) (2000), pp. 261–267

Wong et al., 2012 Y.W. Wong, L.H. Lam, H.C. Tang, Y. Liang, S. Tan, D.T. Yew Intestinal and liver changes after chronic ketamine and ketamine plus alcohol treatment Microsc Res Tech, 75 (9) (2012), pp. 1170–1175

Wu et al., 2012 Q. Wu, M.S. Clark, R.D. Palmiter Deciphering a neuronal circuit that mediates appetite Nature, 483 (7391) (2012), pp. 594–597

Zarate et al., 2006 C.A. Zarate Jr., J.B. Singh, P.J. Carlson, N.E. Brutsche, R. Ameli, D.A. Luckenbaugh, et al. A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression, Arch Gen Psychiatry, 63 (8) (2006), pp. 856–864