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Nitric oxide induced by ketamine/xylazine anesthesia maintains hepatic blood flow during hypothermia

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Abstract

Among the anesthetics influencing the nitric oxide (NO) pathway, ketamine is widely reported in the literature. We researched the variations in blood physiological parameters following ketamine/xylazine- or pentobarbital-induced anesthesia, with particular emphasis on plasmatic NO levels and oxidative stress-related factors. The effects of ketamine on hepatic blood flow during deep hypothermia were also examined. Adult male Sprague–Dawley rats were anesthetized intraperitoneally with ketamine/xylazine or with sodium pentobarbital. Animals underwent serial blood extraction to analyze acid–base balance and lactate levels in blood, as well as NO, MDA, SH groups, and AST levels in plasma samples. We demonstrated that ketamine leads to increased plasmatic NO levels, induces metabolic acidosis, and causes oxidative damage, though without reaching hepatic toxicity. When experimental hypothermia was induced, ketamine affected hepatic blood flow. Based on these results, we suggest that studies on physiological processes involving NO should exercise caution if anesthesia is induced by ketamine.

Keywords: Acid–base balance; Blood flow; Hypothermia; Ketamine; Nitric oxide

Nitric oxide (NO) is a component of the excitatory and inhibitory neurotransmitter pathways involved in anesthesia [1]. In addition, anesthetics have been shown to affect nitric oxide levels [2,3]. Among them, ketamine is widely used in combination with such analgesics as propofol or xylazine [4–6]. The dissociative anesthetic agent ketamine is a non-competitive blocker of the glutamate subtype of the *N*-methyl-D-aspartate (NMDA) receptors [7,8], preventing the neurotransmission of pain. The lack of cardiovascular [9] and respiratory [10] depression at normal doses of ketamine makes it suitable as a general anesthetic. However, some care should be taken when anesthetizing laboratory animals since considerable variation exists among individuals in both the elapsing and depth of anesthesia [6]. In the rat central nervous system, a cyclic GMP accumulation following ketamine-induced anesthesia has been noted, which demonstrates its effects on the nitric oxide pathway [11]. That nitric oxide will lend ketamine such analgesic properties is expected [12]. Although the effects of ketamine-induced nitric oxide in other tissues are somewhat conflicting [13–15], caution should be exercised when dealing with physiological processes in which nitric oxide may play a modulatory role [16]. Our own study focuses on experimental hypothermia, a commonly used therapeutic approach to reduce damage in post-ischemic tissues [17–19]. Since hypothermia is characterized by peripheral vasoconstriction, we hypothesized that this response would vary according to the anesthetic used. Most of the previously published works in deep hypothermia using animal models were performed under barbiturate anesthesia [20,21]. As pentobarbital has not been implicated in the NO pathway, we chose it as our control. The present study was designed to characterize blood physiological parameters following ketamine-induced anesthesia, with particular emphasis on the levels of plasmatic nitric oxide and the related factors of oxidative stress. Moreover, we also addressed the effects of ketamine and pentobarbital anesthesia in hepatic blood flow during deep hypothermia.

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Experimental procedures

Animals and experimental models

Adult male Sprague–Dawley rats (285–300 g body weight) were used. They were housed in a temperature- and humidity-controlled room, on a 12 h light:dark cycle, and fasted overnight. This experimental protocol was approved by the Institutional Committee of Animal Care and Research at the University of Barcelona, in compliance with European Community guidelines.

Animals were anesthetized intraperitoneally with a daily-prepared mixture of ketamine (68 mg kg^{-1} body weight) and xylazine (4.5 mg kg^{-1} body weight) (ketamine group) or with sodium pentobarbital (60 mg kg^{-1} body weight) (pentobarbital group). The doses in both anesthesia protocols (and the time at which the maintenance doses were applied) were the minimum required to produce surgical anesthesia, and were within the range commonly used for laboratory anesthesia in the rat. The required depth of anesthesia was considered to have been attained when corneal reflex and response to painful stimuli were no longer present, according to Gumbleton et al. [22].

Cannulation of the right common carotid artery was performed by insertion of a polyethylene catheter (PE 50), and was completed within the first 15 min following induction of anesthesia.

All anesthetics were provided by Sigma–Aldrich (St. Louis, MO, USA).

Acid–base analyses

Blood samples were obtained in 15 min intervals (0.5 ml) over 1 h, using 1 ml syringes in which air was replaced by heparinized saline solution to avoid alterations in actual blood gas concentrations. The first blood sample was obtained just after cannulation, the others collected at 30, 45, and 60 min. Additional doses of ketamine/xylazine (20 and 1.5 mg kg^{-1}, respectively) were given every 30 min for maintenance. The pentobarbital group, however, required no extra anesthesia [22]. Finally, animals were sacrificed by anesthesia overdose. For every blood sample, arterial pH and partial pressures of arterial blood gases (O_2, CO_2) were measured using the microgas analyzer Radiometer ABL five acid–base laboratory. K^+, Na^+, Ca^{2+}, and Cl^- ion concentrations were measured in blood with the electrolyte analyzer Radiometer EML 100 electrolyte metabolite laboratory (Radiometer, Copenhagen, Denmark). Blood was then used to determine lactate concentration, the rest was centrifuged and the plasma was utilized for biochemical testing.

Biochemical determinations

As an index of plasma NO levels, nitrate and nitrite (NO_x) were monitored using a colorimetric assay kit (Cayman Chemical, USA) previously described [23]. Nitrate was enzymatically reduced to nitrite by incubation with nitrate reductase and NADPH. Nitrite levels were then assayed by Griess reaction and expressed in micro molar. Thiobarbituric acid-reactive substances in plasma (TBARS), which indicate pro-oxidant status, were then determined using the Yagi method, with one slight modification [24]. Butylated hydroxytoluene and EDTA were added to the reaction mixture as antioxidants at a final concentration of 0.01% and 1.3 mol L^{-1}, respectively. TBARS formation was spectrophotometrically measured at 540–620 nm, and the calibration curve was determined using tetraethoxypropane. TBARS values are expressed in terms of malondialdehyde (MDA) concentration in milli molar. Oxidative modification of protein sulfhydryl groups (SH groups) in plasma was quantified in accordance with the method described by Hu [25], using spectrophotometric measurement at 414 nm. Total SH groups are expressed in millimolar. Plasma aspartate aminotransferase (AST) activity was determined using a commercial kit (Merck, Germany), and expressed in units per liter. Blood lactate concentration was measured using a lactate dehydrogenase method with a commercial kit (Boehringer–Mannheim, Germany), and expressed in millimolar.

Hypothermia and hepatic blood flow

A hypothermia protocol, previously described by our group [26], was applied in this study. Anesthetized rats from both groups (ketamine and pentobarbital) were cannulated via the carotid artery for pressure monitoring and blood sampling. Arterial blood pressure and heart rate were continuously recorded on a two-channel polygraph (Model 2006, Letica, Scientific Instruments). A tracheal intubation was also performed. Rats were connected to a volume- and frequency-controlled small-animal respirator (Harvard Apparatus, Kent, UK) using ambient air. They were then placed in a supine position on the cooling table. The cooling apparatus was constructed using one water pumping bath, set to 14 °C. The inlet and outlet of the bath were connected by polyethylene tubes (10 mm inner diameter) and "Y" and "T" connectors. Water flowed through two tubes. One (4 mm inner diameter) was placed under the surgery table, and the other tube (1 mm inner diameter) was bent into a coil of eight turns. These loops were placed inside the abdominal cavity. A flow probe was placed around the portal vein (Transonic Systems NY, USA) and portal blood flow was continuously monitored. Finally, the muscular layer was stitched from skin to prevent heat loss.

Body temperature was calculated as the average of the readings from two thermocouples (2 mm diameter), one inserted next to the carotid artery (core temperature) and the other in the peritoneal cavity near the liver (abdominal temperature). Deep hypothermia was reached 1 h after cooling was triggered. Animals were maintained at 20.65 ± 0.25 °C for 1 h. During this period, ketamine or pentobarbital was not administered, due to the anesthetic effects of hypothermia.