Effects of morphine or ketamine in rats with asphyxial cardiac arrest: a pilot study

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Abstract

Acute hypoxia results in uncontrolled release of glutamate and the consequent stimulation of N-methyl-D-aspartate (NMDA) receptors, which affects the whole ionic homeostasis and finally activates apoptosis of neurons. A potential therapeutic approach to prevent this sequence of events is a blockade of NMDA receptors. Meanwhile, in different models of acute hypoxia, activation of delta-opioid receptors demonstrates significant cardio- and neuroprotective effects with a consequent increase in animal survival. Thus, we aimed to test the effects of Morphine or Ketamine on hemodynamics, acid-base status and early survival in rats after asphyxia cardiac arrest (ACA).

Methods: After instrumentation under anesthesia with Thiopental sodium (60 mg/kg, i.p.), Wistar rats (n=21) weighing between 350–400 g were randomly assigned to three groups where: 1. Morphine 5 mg/kg iv (n=7) was given 10 min before ACA; 2. Ketamine 40 mg/kg iv (n=7) was given 10 min before ACA; 3. Control (n=7), the same amount of NaCl 0,9% iv was given 10 min before ACA. The rats were asphyxiated by corking the tracheostomia tube at the end of expiration for 5 min. Resuscitation was initiated by an injection of epinephrine (0.02 mg/kg, iv), followed by manual thoracic compressions (180 compressions/min) and mechanical ventilation (21% O_2, 80 breaths/min). Invasive mean arterial pressure (MAP) was recorded at the baseline (BL), injections (Inj), every 1 min during ACA (As) and every 5 min in post-resuscitation (PR) period. Blood gas samples were taken at the BL and 10 min at the PR period. Early survival was determined at the 20 min after ACA.

Results: No differences in MAP between the rats was found at the BL period. On the one hand, iv injection of Ketamine significantly reduced MAP before ACA when compared to the Morphine and Control groups. On the other hand, the rats pre-treated with Ketamine got significantly higher MAP during PR period (Fig.1) and had significantly lower production of lactate (Fig.3) when compared to the rats treated with Morphine or only NaCl 0,9%. Six of the seven rats survived at the 20 min after ACA in the Ketamine group while four of the seven and two of the seven rats survived in the Morphine and Control groups respectively (P=0,122) (Fig. 4).

Conclusion: Pre-treatment with Ketamine attenuated significantly disturbances in hemodynamics and acid-base status after ACA, but it did not improve significantly early survival when compared to the rats pre-treated by Morphine or only NaCl 0,9%.

Introduction

Experimental studies demonstrate that opioids can preserve cellular status during acute hypoxia in many organs and tissues including: intestine [1], skeletal muscle [2], myocardium [3,4] and brain [5,6]. In acute hypoxia conditions, Morphine has shown significant neuroprotective effect with a consequent increase in mice and rat survival [7,8]. Meanwhile, it has been also found that high doses of Morphine might inhibit NMDA receptors [9]. Today it is well known that inhibiting of NMDA receptor by Ketamine reduces neuronal apoptosis, attenuates the systemic inflammatory response to tissue injury, and also maintains cerebral perfusion pressure as a result of sympathetic nervous system activation [10-13]. However, to our knowledge nobody has tested the effects of Morphine or Ketamine in rats with acute hypoxia due to cardiac arrest.

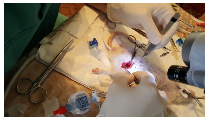
Aims

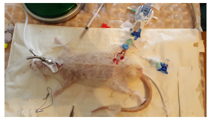
To study effects of pre-treatment with Morphine or Ketamine on hemodynamics, acid-base status, plasma levels of neuron specific enolase (NSE) and s100 calcium binding protein B and early survival in rats after asphyxia cardiac arrest.

Methods and Materials

Ethics: the experimental study with rats was approved by the Animal Care and Use Committee of the Astana Medical University, Astana, Kazakhstan.

Animal Instrumentation: under anesthesia with Thiopental sodium (60 mg/kg, i.p.), each rat was inserted stainless-steel tracheal tube in trachea. The rats were mechanically ventilated with room air and a tidal volume of 8 ml/kg using small animal ventilator (TOPO Dual mode ventilator, Kent Scientific Corp., USA). A 24G central venous catheter (Arrow) was inserted into the right femoral vein for drug administration and taking of blood sampling. A 22G catheter (22G venflon, BD, Sweden) was inserted into the right femoral artery for continuous blood pressure monitoring.





Average time for the instrumentation was about 10 min and all instrumentations in the study were performed by one experienced person.

Methods and Materials (cont.)

After instrumentation and following 10 min pause, all animals were randomly assigned into 3 groups: 1. Morphine group (n=7), where the rats were given iv Morphine 5 mg/kg 10 min before cardiac arrest 2. Ketamine group (n=7), where the rats were given iv Ketamine 40 mg/kg 10 min before cardiac arrest, 3. Control group (n=7), where the rats were given an equal quantity of NaCl 0,9% 10 min before cardiac arrest.

Induction of cardiac arrest (CA): CA was induced by corking of tracheal tube for 5 min and defined as a mean arterial pressure (MAP) bellow 20 mm Hg. Resuscitation was initiated by an injection of epinephrine (0.02 mg/kg, iv) and followed by mechanical ventilation (21% O_2, 80 breaths/min) and manual thoracic compressions (180 compressions/min). Restoration of spontaneous circulation (ROSC) was defined as a return of MAP above 60 mmHg. Ventilation was maintained until spontaneous breathing began. The temperature was kept at 36,5 °C to 37,5 °C by a heating pad during the study. Arterial blood samples were taken at the baseline and at 10 min after the start of resuscitation. MAP was recorded at the baseline, after iv injection of the study drugs or saline, at 1,2,3,4,5 min after induction of CA and at 1,5,10,15,20 min in post-resuscitation period. Levels of neuron specific enolase (NSE) and s100 calcium binding protein B were measured photometrically in rat plasma using ELISA kit (MyBioSource Inc., San Diego, CA, USA). All survived rats were terminated by huge doses of Thiopental sodium at the end of study.

Statistical analysis: Statistical analyses were performed using SPSS software, version 21.0 (SPSS, Inc., Chicago, IL, USA). Data was assessed by two-factor analysis of variance for repeated measurements. If the F value was statistically significant, Scheffé's test was used for post hoc intergroup analysis. P<0.05 was considered to indicate a statistically significant difference.

Results

Pre-treatment of rats with Ketamine resulted in significantly better hemodynamic stability after asphyxial cardiac arrest as compared with two other study groups (Fig. 1). The rats in Ketamine group demonstrated significantly low accumulation of both lactate and hydrogen ions in blood as compared to the rats in Control group (Fig. 2 and 3). As we did not apply oxygen in our experiments, a very high mortality in the Control group was observed (Fig. 4). However, some trends for increasing of survival in both Ketamine and Morphine groups was also observed (Fig. 4). Finally, we did not find any differences in plasma concentration of NSE and s100 calcium binding protein B between the study groups (data not shown).

Figures

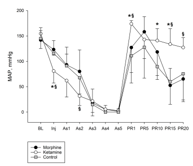


Figure 1. Changes of mean arterial pressure (MAP) in the study groups at baseline (BL), after iv injection (Inj) of the study drugs or saline, at 1,2,3,4,5 min after induction of asphyxia (As) and at 1,5,10,15,20 min in post-resuscitation (PR) period. *P<0.05 between Ketamine and Control groups. §P<0.05 between Ketamine and Morphine groups

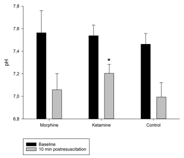


Figure 2. Comparison of pH levels measured at baseline and at 10 min in in post-resuscitation period between the study groups. *P<0.05 between Ketamine and Control groups.

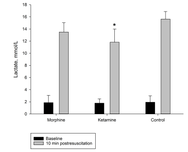


Figure 3. Comparison of Lactate levels measured at baseline and at 10 min in in post-resuscitation period between the study groups. *P<0.05 between Ketamine and Control groups.

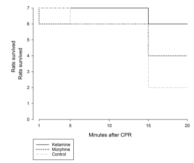


Figure 4. 20-min survival in the Ketamine, Morphine and Control study groups.

Comments and Conclusions

In terms of mortality, our experimental model of asphyxial caridac arrest in rats without application of oxygen mimics closely cardiac arrest situation in humans. After 5 min period of anoxia condition, most of the rats in Control group died during the first 20 min after cardiac arrest. In turn of, trend for increased survival was found in both Ketamine and Morphine groups. Influence of Ketamine on hemodynamic status in our study is very consistent with findings from previous experimental and clinical research works. Further investigations are needed to elucidate whether Ketamine or Morphine play any role in prevention of neuronal injury in experimental animals and humans after cardiac arrest.

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Presentation

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