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Abstract

Anesthetics can affect the structure and biological function of tissues and systems differentially. The aim of the present study was to compare three injectable anesthetics generally used in experiments with animals in terms of the degree of hemolysis and glycogenolysis occurring after profound anesthesia. Twenty-four male Wistar rats (330-440 g) were divided into three groups (N = 8): chloral hydrate (CH), ketamine + xylazine (KX), Zoletil 50® (zolazepam and tiletamine) + xylazine (ZTX). After deep anesthesia, total blood was collected. The liver and white (WG) and red gastrocnemius (RG) muscles were also immediately removed. The degree of serum hemolysis was quantified on the basis of hemoglobin concentration (g/L). Hepatic and muscular glycogen concentrations (mmol/kg wet tissue) were quantified by the phenol-sulfuric method. The CH and KX groups exhibited serum hemolysis (4.0 ± 2.2 and 1.9 ± 0.9 g/L, respectively; P < 0.05) compared to the ZTX group, which presented none. Only KX induced elevated glycogenolysis (mmol/kg wet tissue) in the liver (86.9 ± 63.2) and in WG (18.7 ± 9.0) and RG (15.2 ± 7.2; P < 0.05). The CH and ZTX groups exhibited no glycogenolysis in the liver (164.4 ± 41.1 and 176.8 ± 54.4, respectively), WG (28.8 ± 4.4, 32.0 ± 6.5, respectively) or RG (29.0 ± 4.9; 25.3 ± 8.6, respectively). Our data indicate that ZTX seems to be an appropriate general anesthetic for studies that seek to simultaneously quantify the concentration of glycogen and serum biochemical markers without interferences. ZTX is reasonably priced, found easily at veterinary markets, quickly induces deep anesthesia, and presents a low mortality rate.

Key words: General anesthetic; Rats; Glycogenolysis; Hemolysis; Zoletil; Ketamine

Introduction

Studies on animals often involve the use of various anesthetic types for sample collection, i.e., inhaled agents or agents injectable by the intraperitoneal, intramuscular or intravenous route. Since the anesthetics can affect the structure and function of organs and biological systems in different manners, some effects should be further elucidated. For example, hemolysis is a pre-analytical interfering factor in clinical chemistry (1). Serum concentrations of Hb above the threshold values can interfere with various biochemical analyses. For human serum, the threshold values are: lactate dehydrogenase (Hb >0.2 g/L), aspartate aminotransferase, potassium and acid phosphate (Hb >1.5 g/L), creatine kinase (Hb >2.5 g/L), alanine aminotransferase (Hb >3.4 g/L), bilirubin (Hb >0.8 g/L), alkaline phosphatase and albumin (Hb >1.5 g/L), and gamma-glutamyltransferase (Hb >3.0 g/L) (2). Hepatic and muscular glycogen concentrations can also be depleted depending on the anesthetic used.

An injectable general anesthetic frequently used in studies with animals is chloral hydrate, which, however, produces cardiovascular and breathing depression and can cause blood acidosis (3). The barbiturate pentobarbital is one of the most widely used general anesthetics in studies with animals, its main advantage being its low cost. Pentobarbital has a low analgesic effect and induces breathing depression, also frequently halting breathing before the beginning of surgery (3). The effect of thiopental and pentobarbital administration on biochemical parameters has not been fully explained and there are studies showing interference with...
glycogen metabolism after their administration (4,5).

Ketamine and tiletamine are two injectable dissociative general anesthetics. Ketamine has analgesic properties, but also seems to affect the cardiovascular system (6). Tiletamine is a more potent anesthetic than ketamine and is used in combination with zolazepam, which is similar to diazepam (7). These two dissociative anesthetics are usually employed in combination with xylazine, which has analgesic, sedative and muscle relaxant effects (7).

The aim of the present study was to compare three injectable anesthetics commonly used in experiments with animals and to examine the degree of serum hemolysis and glycogenolysis in order to determine which would be appropriate for use in studies that need to simultaneously quantify tissue glycogen concentrations and serum biochemical markers without interferences.

Material and Methods

Animals

Twenty-four 80-day-old male Wistar rats weighing 330 to 440 g were housed at 22°C under an inverted 12:12-h light-dark cycle (18:00-6:00 lights on), with free access to laboratory rat chow and water. The experimental protocols were approved by the Ethics Committee for Animal Experimentation of the Universidade Estadual de Campinas (UNICAMP) (Protocol #1565-1).

The anesthetic concentrations used were tested in a pilot experiment to guarantee that the time to induction of deep anesthesia did not exceed 15 min. On the day of the experiment, the animals were divided at random into three groups, which were subjected to different anesthetic protocols: chloral hydrate (CH; Reagen Quimibrás Chemical Industry S.A., Brazil), 600 mg/kg weight corresponding to 3 mL/kg weight, xylazine hydrochloride (CH; Anasedan, Vetbrands), 11 mg/kg weight corresponding to 0.48 mL/kg weight, ip, N = 8; ketamine hydrochloride (KX; Anasedan, Vetbrands), 100 mg/kg weight corresponding to 1.16 mL/kg weight + xylazine hydrochloride (KX; Anasedan, Vetbrands), 11 mg/kg weight corresponding to 0.48 mL/kg weight, ip, N = 8; Zoletil 50® (50 mg/kg weight corresponding to 1 mL/kg weight) + xylazine hydrochloride (ZTX), 11 mg/kg weight corresponding to 0.48 mL/kg weight, im, N = 8. The Zoletil 50® (Virbac of Brazil, Brazil) anesthetic used contained a 1:1 ratio (125:125 mg) of tiletamine hydrochloride and zolazepam hydrochloride. The rats were weighed before the anesthesia for calculation of the anesthetic volume to be injected.

All animals were sacrificed at a similar time, from 9:00-11:00 h, to avoid confounding due to possible hormonal interference with glycogen metabolism (8).

Blood and tissues collection and analysis

The animals were anesthetized with their respective general anesthetics and kept in individual cages until deep anesthesia was achieved. The corneal, auricular, abdominal clamp, tail clamp, and pedal clamp reflexes were monitored. After complete anesthesia was achieved, closer to 5 min for KX and ZTX groups and 15 min for the CH group, the surgery was started with the opening of the thoracic cavity. Total blood was collected by heart puncture in the left ventricle, stored in Vacuette tubes (Greiner Bio-One, Brazil) with gel and immediately centrifuged at 1800 g at 4°C for 10 min prior to serum collection. Immediately after blood removal, 35 to 50 mg of liver samples and different portions of the white (WG) and red (RG) gastrocnemius muscle were collected. The tissues were weighed, placed in microtubes, immediately frozen in liquid nitrogen and kept at -80°C for one week. According to previous studies from our laboratory (data not shown), this period of time does not interfere with glycogen concentrations.

The extent of serum hemolysis was determined on the basis of Hb concentration (g/dL) using the Hb-cyanide spectrophotometric method with the Drabkin reagent (9). Hepatic and muscle glycogen was also quantified using the spectrophotometric phenol-sulfuric methods proposed by Lo et al. (10).

Table 1. Glycogen concentration in hepatic and muscle tissues following treatment with chloral hydrate, ketamine plus xylazine, or zoletil plus xylazine.

<table>
<thead>
<tr>
<th></th>
<th>CH</th>
<th>KX</th>
<th>ZTX</th>
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<tbody>
<tr>
<td>Liver</td>
<td>164.4 ± 41a</td>
<td>86.9 ± 63.2b</td>
<td>176.8 ± 54.4a</td>
</tr>
<tr>
<td>White gastrocnemius</td>
<td>28.8 ± 4.4a</td>
<td>18.7 ± 9.0b</td>
<td>32.0 ± 6.5a</td>
</tr>
<tr>
<td>Red gastrocnemius</td>
<td>29.0 ± 4.9a</td>
<td>15.2 ± 7.2b</td>
<td>25.3 ± 8.6a</td>
</tr>
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</table>

Data are reported as means ± SD of tissue glycogen concentration (mmol/kg wet tissue) for 8 animals in each group. CH = chloral hydrate (600 mg/kg weight corresponding to 3 mL/kg weight, ip); KX = ketamine hydrochloride (100 mg/kg weight corresponding to 1.16 mL/kg weight) + xylazine hydrochloride (11 mg/kg weight corresponding to 0.48 mL/kg weight, ip); ZTX = Zoletil 50® (50 mg/kg weight corresponding to 1 mL/kg weight) + xylazine hydrochloride (11 mg/kg weight corresponding to 0.48 mL/kg weight, im). Complete anesthesia was achieved closer to 5 min for KX and ZTX groups and 15 min for the CH group. Different letters represent significant differences among the groups (P < 0.05; ANOVA followed by the Tukey post hoc test).

Statistical analysis

Statistical significance was set at P < 0.05. One-way analysis of variance (ANOVA) followed by the Tukey post hoc test was performed using the statistical program Graphpad Instat 3 (GraphPad Software, Inc., USA).

Results

Measuring Hb concentration in each anesthetized group, the CH and KX anesthetics induced serum hemolysis, with CH having a higher hemolytic action (CH: 4.0 ± 2.2; KX: 1.9 ± 0.9 g/L; P < 0.05). The serum from the ZTX group did not present hemolysis.

Table 1 presents the hepatic and muscular glycogen concentrations.
concentrations of both the white and red portion of the gastrocnemius muscle (mean ± SD) in each anesthetized group. Only the KX anesthetic induced marked glycogenolysis in liver as well as in gastrocnemius muscle (P < 0.05). We found no effect of CH or ZTX on tissue glycogen concentrations.

Discussion

In the present study, different anesthetics applied to Wistar rats were compared regarding the degree of serum hemolysis and tissue glycogenolysis occurring after deep anesthesia. There are several studies in the literature comparing the cardiovascular effects of different anesthetics (7,11). However, few of them analyze their effects on blood biochemical parameters, which could suffer from interference (4). The present study did not quantify any biochemical parameters and therefore we cannot discuss possible interference. Even so, our data facilitate the choice of an anesthetic that does not cause hemolysis (such as ZTX) for studies measuring biochemical parameters.

The CH anesthetic did not induce glycogenolysis, in agreement with the study of Field et al. (3). However, it did elicit a greater hemolytic effect. The average Hb concentration in the CH group samples was visible and above the threshold values of interference for some biochemical analyses (2). It is known that CH is metabolized quickly as trichloroethanol and trichloroacetic acid in the blood and liver. These metabolites are highly toxic to cells (12). Trichloroacetic acid is a strong acid protein precipitant that causes lipid peroxidation of the cell membranes (13). Lipid peroxidation may be the mechanism underlying the marked hemolysis occurring in the red blood cells of animals anesthetized with CH.

The KX anesthetic produced about half the hemolysis of CH. On the other hand, KX produced a sizable muscular and hepatic glycogenolytic reduction compared to CH. Ketamine seems to disturb the integrity of the red blood cell membrane by interacting directly with the glucose carrier (GLUT-1). This interaction inhibits glucose uptake, which could render the cells hyotonic, precipitating hemolysis (14). The glycogenolytic effect may be related to increasing catecholamine release due to activation of the sympathetic nervous system that seems to occur soon after the administration of this drug (15). It is not possible to rule out a direct action of KX on some enzymes involved in glycogen metabolism (15).

Xylazine is an alpha-2 adrenergic agonist that can cause hyperglycemia in some animals, contributing to a glycogenolytic action when combined with ketamine. Rodrigues et al. (16) found much higher values of blood glucose in rats anesthetized with KX when compared to rats anesthetized with CH, suggesting hepatic glycogenolysis. In contrast, Musch et al. (17) did not observe alterations in glycogen concentration after KX administration. However, the method used to induce deep anesthesia involved a lower dose of anesthetic (ketamine: 50 mg/kg weight and xylazine: 3 mg/kg weight), combined with gas maintenance (70% nitric oxide and 30% oxygen). It is thus likely that the lower concentration of anesthetic resulted in less activation of the sympathetic nervous system.

ZTX is a combination of tiletamine-zolazepam-xylazine and proved to be the most appropriate of the anesthetics for studies that require the simultaneous quantification of the concentration of glycogen and serum biochemical markers without interferences. Although tiletamine alone activated the sympathetic nervous system and tended to induce convulsive activity (18), it was efficient when administered with zolazepam, which possesses anticonvulsive, anxiolytic, sedative-hypnotic, and muscle relaxant properties (7,18). When administered together, these anesthetics induced neither hemolysis nor glycogenolysis immediately after the deep anesthesia. This combination of compounds has been used as a tranquilizer to immobilize wild animals (7,19). Furthermore, many studies have already demonstrated its effectiveness in anesthetizing rats and other animals (7,18-20). In a comparative study of ketamine-xylazine, pentobarbital and tiletamine-zolazepam, the last caused fewer cardiovascular effects in rats when compared to the others (11). In another study, it was shown that ketamine-xylazine resulted in faster breathing rates and higher arterial O2 partial pressure than tiletamine-zolazepam-xylazine (20).

In the present study, we used the commercial anesthetic Zoletil 50®, which is a combination of tiletamine-zolazepam and xylazine, to cause deep anesthesia, analgesia, sedation, and efficient muscle relaxation prior to surgery (7,19,20). This combination also decreased the final cost, because it reduced the Zoletil 50® concentrations required.

ZTX seems to be an appropriate anesthetic to be used in research with laboratory animals. The cost of this method is reasonable, especially since it does not require an inhaler. Moreover, the compound is commonly found at veterinary markets, can be applied with ease, induces deep anesthesia quickly, and presents a low mortality rate.

References
