The impact of an anaesthetic protocol on blood and renal acid-base parameters during a study of ECF volume expansion in a small group of clinically healthy rats

***P. A. Walsh & +D.J. O'Donovan**

*Department of Physiology, School of Medicine, RCSI - MUB, Building No. 2441, Road 2835, Busaiteen 228, Kingdom of Bahrain.

+Department of Physiology, National University of Ireland Galway (NUIG), Ireland.

Corresponding author: Dr. Patrick Walsh, Department of Physiology, School of Medicine, RCSI Bahrain, Building No. 2441, Road 2835, Busaiteen 228, Kingdom of Bahrain.

Email: pwalsh@rcsi-mub.com

Abstract

The purpose of this study was to assess the suitability of an anaesthetic protocol for the investigation of blood and urinary acid – base parameters in a group of clinically healthy rats.

Six rats were lightly anaesthetized with di-ethyl ether. Intraperitoneal injections of sodium pentobarbitone were given to maintain the anaesthesia. A solution containing 0.9% saline and 3% ethanol was infused at a rate of 87µl.min^{-1} via an indwelling cannula, which had been placed in the jugular vein.

Arterial blood samples were collected from the carotid artery towards the termination of the infusion period. ABG analysis gave the following data:

pH = 7.26 ± 0.04 (n=6), pCO2 = 56 ± 4 mmHg (n=6), and [HCO^-_3] = 24 ± 3 mmoles.Litre^{-1} (n=6).

Urine was collected at timed intervals from the left kidney through a cannula, which had been positioned in the ureter. Urinary specimens were analysed for flow rate and ammonium concentrations. Very low urinary flow rates were observed, which did not increase significantly over the course of the time frame of the infusion. However, urinary ammonium increased significantly from 4.59 ± 2.70 to a maximum excretion rate of 15.21 ± 12.94 µmoles.hr^{-1} (n=6), $p < 0.05$.

Considering the development of hypercapnia, respiratory acidosis, and increased urinary ammonium excretion during the course of the study, the anaesthetic regimen that was used was deemed to be an unsuitable modality for the study of either respiratory or renal function in the rat.

Keywords: Rat, renal, acidosis, ammonium, anaesthetic protocol.

1. Introduction

Animal experiments are necessary for the better understanding of physiological processes, diseases, and for the development of new therapeutic strategies. The rat (*Rattus norvegicus*) is one of the most widely used species in biomedical research. Experimentation often requires the use of anaesthetic protocols to facilitate animal immobilization and to reduce stress or pain (Furtado & Andrade, 2013). Anaesthesia in laboratory animals has been defined as a state of unconsciousness, analgesia, muscle relaxation, and areflexia (Kohn, Wixson, White, & Benson, 1997). An ideal anaesthetic should be easy to dispense, produce rapid and pain-free restraint, have minimal toxicity, and be reversible and safe for both animals and operators. While a broad range of anaesthetic agents are currently clinically available, we have concentrated in this study on the determination of the suitability of di-ethyl ether and sodium pentobarbitone as anaesthetic agents for the investigation of renal acid-base changes in the rat.

Ether is a colourless, volatile and highly inflammable liquid with a characteristic pungent smell. In the presence of oxygen, high concentrations of ether vapour can explode with potentially devastating consequences. Because ether has a high blood/gas solubility, is an irritant to the respiratory tract, and has a low potency, the induction – of, and recovery - from anaesthesia is relatively slow by comparison with other volatile anaesthetic agents. Despite its many shortcomings, ether is a relatively safe anaesthetic primarily because, with overdose, respiratory depression precedes serious cardiac depression (Bovill, 2008). However, because of its explosive properties and toxicity to both humans and animals, the use of di-ethyl ether, as an anaesthetic in small animal laboratory investigations, has diminished in recent times. Currently, the use of ether as an anesthetic agent in animals is not permitted without strong scientific justification and approval by the IACUC (Silverman, Suckow, & Murthy, 2014).

Intraperitoneal injection of sodium pentobarbitone has long been accepted as a humane method in rodent anaesthesiology. Sodium pentobarbitone is a short-acting oxybarbiturate, inducing a sleep time of 30 – 60 minutes in the rat. Its main adverse effects, however, are respiratory and cardiovascular depression (Field, White, & Lang, 1993; Vidt, Bredemeyer, Sapirstein, & Sapirstein, 1959).

With the objective of identifying a suitable anaesthetic protocol for the investigation of renal function in the anaesthetized, acidotic rat, an experiment was conducted on a control group of healthy animals who were lightly etherized followed by the intraperitoneal injection of low and periodic doses of sodium pentobarbitone to maintain the anaesthesia over a 2 – 3 hour time frame while a solution containing 0.9% saline and 3% ethanol (Leonard & Orloff, 1955) was being infused through an indwelling jugular vein cannula.

2. Methods

2.1 **Source & ethics governing the use of experimental animals:** *Animal husbandry:* Clinically healthy, male albino Sprague-Dawley rats, each weighing 370 – 453 grams (n=6), were used in all experiments. They

were housed in solid-bottom polyethylene caging with bedding, in an environmentally controlled room (20±1^{0}C, 35 – 60% relative humidity, and 12:12 light-dark cycle). The animals were allowed free access to water and rodent diet (Bioresources unit, TCD, Dublin, Ireland).

Ethical approval: The research work described in this publication was carried out in the Physiology laboratories at the National University of Ireland Galway (NUIG), Ireland.
A license to undertake the animal experiments was granted by the Department of Health (Public Health Division), Hawkins House, Dublin 2, Ireland.
The data for this study was collected during a period when it was permitted to use di-ethyl ether as a volatile anaesthetic in animal experiments.

2.2 Pre-operative preparation:

An open drop method was used to deliver diethyl ether to the rats, who had been fasted overnight. Briefly, cotton wool soaked in an aliquot (approx. 5ml) of diethyl ether, was placed in a glass desiccator, under a screen to avoid any skin irritation to the rat caused by contact with the soaked cotton. Each rat was monitored after being placed inside the desiccator with a tightly closed lid. A reduction in the animal's respiratory rate and loss of the righting reflex were indicative of a state of impending anaesthesia, which occurred approximately 3 – 4 minutes after diethyl ether exposure. The rat was immediately removed from the desiccator as soon as these signs were observed. A nose cone, containing a cotton wool ball soaked in 1ml of di-ethyl ether, was placed over the animals nostrils for approximately 15 minutes. In the meantime, the rat was now given intraperitoneal pentobarbital (Nembutal, Abbot Laboratories: 5% solution; 30mg. Kg^{-1} body weight), supplemented with small i.v. bolus doses as required. We determined that surgical anaesthesia had been successfully induced when the pedal withdrawl reflex was absent and we had achieved muscle relaxation. The anaesthetized rat was placed in dorsal recumbency, on a small operating table, and secured in position using four limb restraining robes. Rectal temperature was maintained through-out the surgery at 37^{0}C, using a homoeothermic blanket system with a flexible measuring thermistor probe. Heart and respiratory rates along with blood gases were regularly monitored during the course of surgery.
At the end of the study, the animals were sacrificed by exsanguination from the abdominal aorta into a heparinized syringe.

2.3 Infusion of solutions:

Infusion was made through the left jugular vein through an in situ P.E. 10 cannula, which was connected to a loaded 60ml syringe, positioned on a Harvard I.V. syringe pump (model 2681).
A solution containing 0.9% NaCl & 3% ethanol was infused at a rate of 87 µl.min^{-1} over a 2 - 3 hour period.

2.4 Collection of urine for the measurement of urinary parameters:

After shaving away the fur and sterilizing the caudal area of the abdomen using 0.5% Hibitane in 70% isopropyl alcohol solution, the bladder was exposed and exteriorized through a 2cm suprapubic midline abdominal incision. Under stereomicroscopic observation, the left ureter was isolated and punctured with a syringe needle. A beveled P.E. 10 cannula was inserted into the ureter through the artificially created orifice. The cannula was ligated in position. Urine was allowed to pass into pre-weighed 400µl Eppendorf cups, each of which contained 50µl 0.1M H_2SO_4. The urine volumes were determined gravimetrically.

2.5 Measurement of urinary ammonium:

Urinary specimens were assayed for ammonium by a modification of the alkaline phenate reaction described in the literature, as follows:
5.0 ml of phenol-pentacynonitrosyloferate (50 grams phenol and 0.25 grams sodium nitroprusside per litre) was added to each of the labelled 15 ml test tubes. 20µl of each urine sample / standard (20µmoles.ml^{-1}) / blank, to be analysed, was pipetted (in duplicate) into the phenol reagent and the mixture immediately vortexed. Subsequently, 5.0 ml of alkaline hypochlorite solution (20 grams NAOH pellets and 43 ml 5.25% NaOCl per litre) was added. The samples were incubated at 37^{0}C for 40 minutes. After equilibrating to room temperature, the absorbance at 625 nm was spectrophotometrically determined. The time interval between the addition of the two reagents to the samples / standards / blanks was maintained constant for the purpose of good intra-assay replication.

2.6 Arterial blood sampling

Arterial blood was collected from the left common carotid artery through an in situ P.E. 50 cannula, containing a luer lock. The cannula was filled with a heparinized saline solution (25 U.ml^{-1} in 0.9%Nacl) between samplings. All blood samples were collected anaerobically from the cannulated artery in a 1ml plastic disposable syringe whose dead space had been pre-filled with a sterile, neutral, isotonic heparin solution (5000 I.U.ml^{-1}).

2.7 Measurement of blood acid-base parameters:

Blood pH and pCO_2 measurements were made using a Radiometer BMS-2 micro-electrode assembly with an acid-base analyser. Bicarbonate concentrations were calculated using the Henderson-Hasselbach equation.

2.8. Data analysis

Statistical analysis and presentation of the data was done using GraphPad Prism version 6.00 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com. Endnote x7.5 was used to compile the reference list.

3. Results

A small control group of six anaesthetized rats were infused with a solution containing 0.9% NaCl and 3% ethanol, as described in Methods.

3% ethanol was added to the infusion to counteract the antidiuretic effects of the trauma of surgery. The infusion was commenced as soon as possible following induction of anaesthesia and subsequent cannulation of the jugular vein. Figure 1 captures the volumes of solution that were infused over the time frame of the study. Approximately, a volume of solution equivalent to the plasma volume of each animal had been infused by the termination of the infusion period. During the course of the experimental period, blood and urinary specimens were collected.

Blood was sampled from the carotid artery towards the end of the infusion experiment and analysed immediately. The following data were obtained:

pH = 7.26 ± 0.04 (n=6), pCO2 = 56 ± 4 mmHg (n=6), $[HCO^{-}_{3}]$ = 24 ± 3 mmoles.Litre^{-1} (n=6).

Urine was collected from the left kidney at 15 minute intervals during the course of the infusion. The volumes of the urinary specimens were recorded. While the left kidney urinary flow rate increased from 0.07 ± 0.04 to 0.13 ± 0.09 ml.Hr^{-1} (n=6), the increase was found to be insignificant ($p > 0.05$). (Figure 2).

Using the Berthelot assay to measure ammonium in the urinary specimens, it was found that ammonium excretion increased significantly from 4.59 ± 2.70 to a maximum excretion rate of 15.21 ± 12.94 µmoles.hr^{-1} (n=6), $p< 0.05$ (Figure 3) over the course of the experiment.

4. Discussion

Research was being planned to investigate the effects of a range of systemically administered compounds on blood and renal acid-base parameters in a group of clinically healthy rats. Because the observations were to be conducted over a relatively short time frame of 2 – 3 hours, a protocol which facilitated the accurate and continuous monitoring of both the blood and urinary acid-base status of the animals was required. At the time of this study, the range of implantable devices and equipment currently available from emka technologies and other sources (Kebler, McDonald, & Cadnapaphornchai, 1985), which permit remote analysis on conscious animals, were not at our disposal. Furthermore, a rodent restraining device such as manufactured by Harvard Apparatus were not accessible. The only alternative was to use anaesthetised animals. Thus, the selection of an anaesthetic regimen that interfered minimally with the acid-base status of the animals was an essential part of the experimental design.

The rats that were available to study were quite large in size, as reflected in their body weights. Unlike smaller sized animals, they were difficult to handle and it was not possible to safely administer intraperitoneal injections. For this reason, di-ethyl ether was selected to lightly anaesthetize the animals, prior to the administration of maintenance doses of sodium pentobarbitone.

Even though great care was taken in the selection of a relatively low dosage of this barbiturate, the intraperitoneal administration of this anaesthetic to the animals, who had been previously subjected to di-ethyl ether inhalation, resulted in a depression of respiration, leading to respiratory acidosis due to hypercapnia; this is clearly evident when comparing the acid-base data of the anaesthetized animals with reference values reported in the literature for the un-anaesthetised rat (Brun-Pascaud, Gaudebout, Blayo, & Pocidalo, 1982) .This change in extracellular acid-base status along with ECF volume expansion induced by saline infusion would have provided the stimuli for the enhanced secretion and excretion of the copious amounts of urinary ammonium that was detected in the urinary specimens (Adler, Fraley, & Zett, 1981; Barker, Singer, Elkinton, & Clark, 1957).

Despite volume expansion and the incorporation of ethanol in the infusate (Leonard & Orloff, 1955), very low urinary flow rates, relative to those documented in the literature (Adler et al., 1981), were observed in this study. This more than likely was due to stress-induced hormonal secretion, caused by the inhalation of di-ethyl ether (Knigge et al., 1999; Tinnikov, 1999; van Herck et al., 1991; Zardooz, Rostamkhani, Zaringhalam, & Faraji Shahrivar, 2010; Zelena, Kiem, Barna, & Makara, 1999).

In conclusion, the results indicate that the combination of di-ethyl ether and sodium pentobarbitone is not a suitable anaesthetic choice for either respiratory or renal investigations in the rat. Any measurements that have been made using such an anaesthetic modality do not correctly describe the basal condition.

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Appendices

Declarations

Competing interests: None.

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Guarantor: PW

Contributorship: PW & DJO'D compiled and wrote this manuscript

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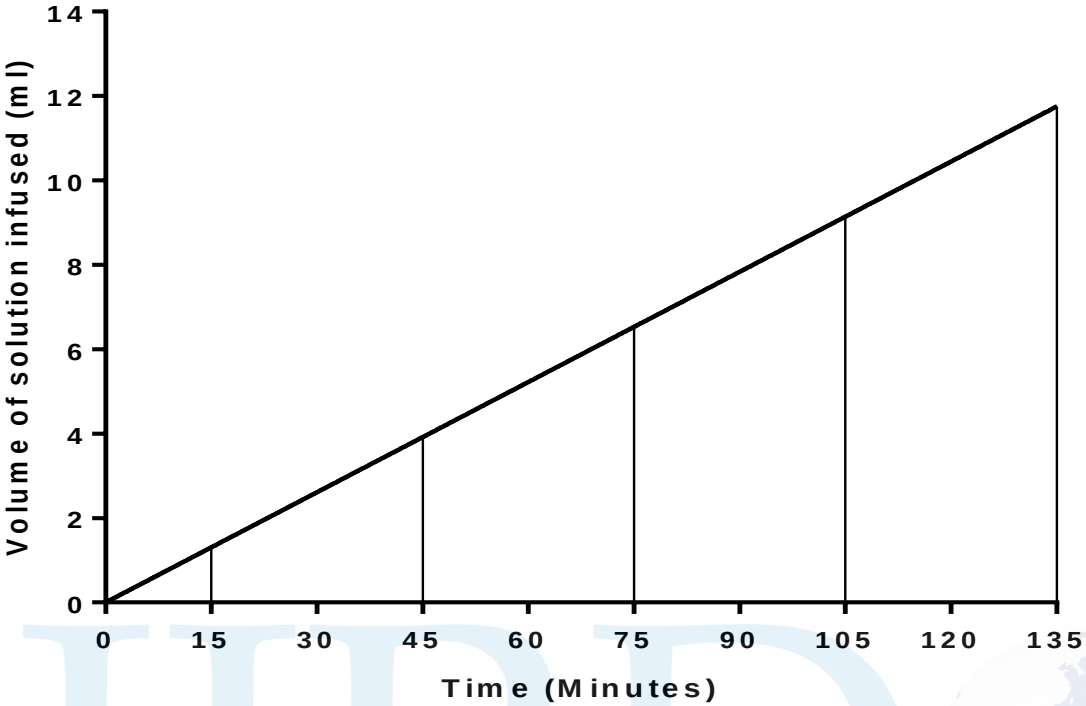
Figure Captions

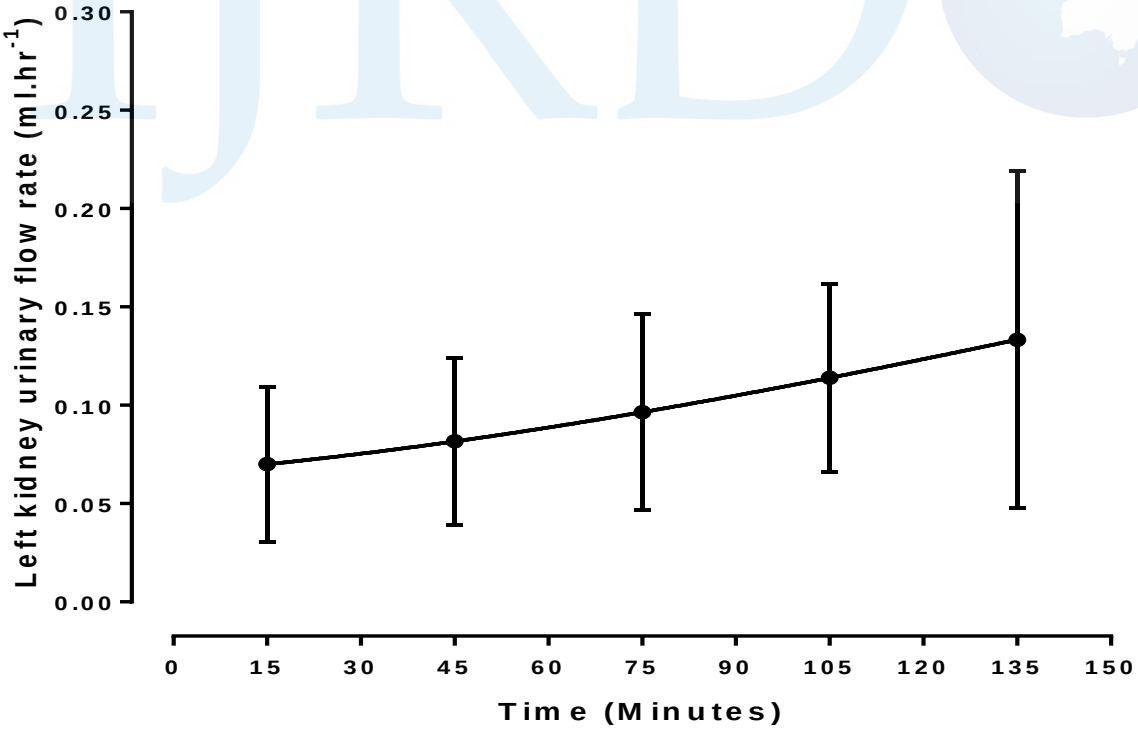
Fig. 1. The time course of the volume of solution infused.

Fig. 2. Left kidney urinary flow rates during the course of infusion of a solution containing 0.9 % NaCl & 3% ethanol.

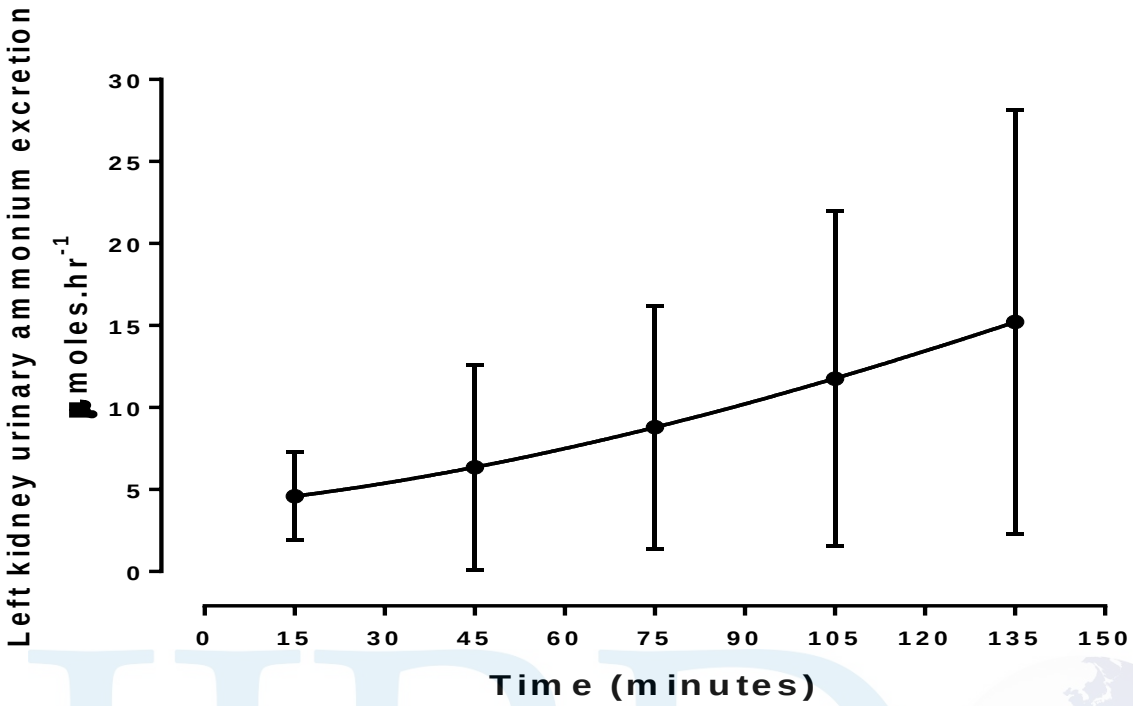
Fig.3. Left kidney urinary ammonium excretion rates during the course of infusion of a solution containing 0.9 % NaCl & 3% ethanol.


Volume of solution infused (ml)
0
2
4
6
8
10
12
14
0
15
30
45
60
75
90
105
120
135
Time (Minutes)

1


Left kidney urinary flow rate (ml.hr-1)
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0
15
30
45
60
75
90
105
120
135
150
Time (Minutes)

2


Left kidney urinary ammonium excretion
moles.hr-1
30
25
20
15
10
5
0
0
15
30
45
60
75
90
105
120
135
150
Time (minutes)

1