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Acid-base status and cardiovascular function in pigs and rats anaesthetized with alpha-chloralose and nitrous oxide

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Introduction

From an ethical point of view acute non-recovery animal experiments are preferable to chronic animal experiment. However, if the interpretation of the results from acute experiments are to be reliable, the animal must be anaesthetized with a drug that induces proper anaesthesia and at the same time has minimal effects on physiological parameters such as cardiovascular parameters and acid-base balance of the blood.

Alpha-chloralose is a commonly used anaesthetic in acute animal experiments, where stable cardiovascular parameters are important. However, it is not known how alpha-chloralose induces anaesthesia, and there appear to be considerable species differences in the anaesthetic action of the drug.

Croft (1964) expressed doubt that alpha-chloralose was in fact an anaesthetic. This view was supported by results showing increased heart rate and arterial blood pressure in animals undergoing extensive surgery, exclusively anaesthetized with alpha-chloralose (*Bass & Buckley* 1966, *Charney et al.* 1970). Chronically instrumented animals, on the other hand, showed no changes in heart rate or blood pressure when anaesthetized with alpha-chloralose (Cox 1972). Alpha-chloralose appears to be a hypnotic rather than an anaesthetic drug (*Halkola et al.* 1974).

To avoid possible pain perception in animals anaesthetized with alpha-chloralose, an additional analgesic compound must be added if alpha-chloralose is to be used during acute experiments. Morphine, urethane and barbiturate (*Harley et al.* 1968), barbiturate (*Van Citers et al.* 1964, *Ledsome et al.* 1971) or Halothane (*Svendsen et al.* 1990) have been suggested. However, it may influence various physiological parameters when alpha-chloralose is combined with other compounds.

Nitrous oxide has proven analgetic properties, minor hypnotic properties and limited effects on physiological parameters. The aim of the present study was to examine if the combined effect of alpha-chloralose and nitrous oxide could maintain surgical anaesthesia in pigs and rats without changing cardiovascular and respiratory parameters.

Materials and methods

Animals: Experiments were performed on twelve female Danish Landrace pigs, weighing 22-30 kg, and five conventional male Sprague Dawley rats (Mol:SPRD), weighing 390-545 g. Pigs were kept in solid floor pens with straw as bedding, and were fed a standard pig diet. Rats were housed in Macrolon type IV cages with Hundstrup 20-40 OMK wood-shavings as bedding, and were fed Altromin diet nr. 1324 ad libitum. Tap water was constantly available for both species. The temperature was 20-22 °C with a relative humidity of 55-65% and 20 air changes per hour. The photoperiod was 12 hours, with lights on from 6 am to 6 pm.

Anaesthesia

The pigs were sedated with 4 mg/kg i.m. azaperone (Sedaperone vet. 4%, Janssen Pharmaceutica, Beerse, Belgium). Anaesthesia was induced 15 minutes after sedation with 5 mg/kg i.p. metomidate hydrochloride (Hypnodil vet. 5%, Janssen Pharmaceutica, Beerse, Belgium). After another 15 minutes a 20 G Venflon intravenous cannula was placed in an ear vein, and metomidate hydrochloride was infused until sufficient relaxation was obtained (5-10 mg/kg). The trachea was intubated and artificial respiration (Servo ventilator 900, Elena Schönander, Sweden) with 50% oxygen and 50% nitrous oxide was applied at approximately 150 ml/kg/min. After 45 minutes, anaesthesia was maintained by i.v. infusion of saline with 0.53% alpha-chloralose (Sigma Chemical, St.. Louis, Mo., USA). Over a 30 minute period 180 mg/kg alpha-chloralose was administered.

In the rats, anaesthesia was induced with 4.0 mg/kg s.c. midazolam (Dormicum, Hoffmann-La Roche, Basel, Switzerland), followed by 0.30 ml/kg i.m. fentanyl-fluanisone (Hypnorm, Janssen Pharmaceutica, Beerse, Belgium). The trachea was intubated and artificial respiration (Harvard Rodent Ventilator 680, Newport Beach, Calf., USA) with 50% oxygen and 50% nitrous oxide was applied at approximately 355 ml/kg/min. The jugular vein and carotic artery were cannulated. After 60 minutes anaesthesia was continued with a 1% alpha-chloralose (Sigma Chemical, St. Louis, Mo., USA) solution in saline, administered in the jugular vein with an infusion pump (Terufusion STC-521, Tokyo, Japan). The average initial dose was 97 mg/kg; the total average dose administered in small increments over 8 hours was 288 mg/kg.

Experimental procedures

In the pigs, the cephalic vein was cannulated for infusion with saline. The femoral artery was cannulated for withdrawal of blood samples and continuous recording of the arterial blood pressure. An ECG displayed the heart rate. Heart rate and mean arterial blood pressure were recorded every 15 minutes, beginning 150 minutes after the initiation of alpha-chloralose infusion. Arterial blood samples of 2 ml were collected at 45 minute intervals beginning 105 minutes after alpha-chloralose infusion, and analyzed at 37 °C for pH and pCO_2 (BMS 2 MK Blood Micro System, Radiometer, Copenhagen, Denmark).

In the rats, blood pressure from the carotic artery was recorded. An ECG displayed the heart rate. Heart rate and mean arterial pressure were recorded at intervals of 15 minutes, beginning 15 minutes after the initiation of the alpha-chloralose infusion. Arterial blood samples of 0.7 ml were taken and analyzed at 37 °C for pH and pCO_2 (BMS 2MK Blood Micro System, Radiometer, Copenhagen, Denmark). The first sample was taken at 15 minutes, the second at 85, the third at 195 and the last sample at 435 minutes after alpha-chloralose infusion. The level of anaesthesia was monitored by observing the electrocardiogram (ECG) and blood pressure.

Calculations and statistical analysis

The mean arterial pressure (MAP) was calculated from: $MAP = (P_{syst}-P_{diast})/3 + P_{diast}$

The cardiovascular parameters heart rate (HR), mean arterial pressure (MAP) and heart rate-pressure product (HRPP), and the respiratory parameters arterial pH, pCO_2 and calculated base excess (BE) were compared to the normal values of the two species. Normal values were taken from *Svendsen & Carter* 1985 and 1989 (table 1).

Results are expressed as medians and 80% confidence interval for the median. If two confidence intervals do not overlap, the likelihood that the experimental results are different from the reference values is $p<0.01$.

Results

In all animals a satisfactory level of surgical anaesthesia was obtained by alpha-chloralose and nitrous oxide. Figure 1 shows the mean arterial blood pressure in pigs and rats from 150 to 375 minutes and 15 to 435 minutes respectively after the begin-

TABLE 1: Reference values for cardiovascular and respiratory parameters of rats and pigs (*Svendsen & Carter* 1985 and 1989).

	Rat	Pig
HR (min^{-1})	432±58	83±15
MAP (mmHg)	125±6	97±14
HRPP (10^3 mmHg/min)	54 (calc.)	8.05 (calc.)
pH	7.43±0.03	7.43±0.03
pCO_2 (mmHg)	38.3±2.7	40±3
BE (mM)	1.4±1.7	2.2±2.5

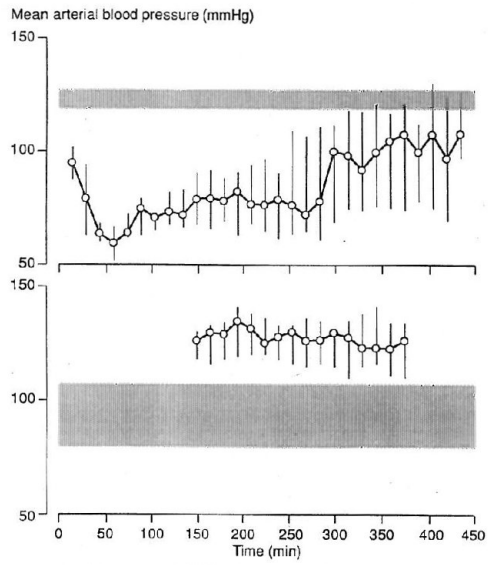

Figure 1. Mean arterial blood pressure in rats (upper graph, n=5) and pigs (lower graph, n=12 (n=6 from 315 min)) while anaesthetized with alpha-chloralose and nitrous oxide. Horizontal bands delineate reference values for conscious animals (80% confidence interval for the median). Values are median and 80% confidence interval. If the 80% confidence interval of values and reference values do not overlap they are significantly different ($p<0.01$).

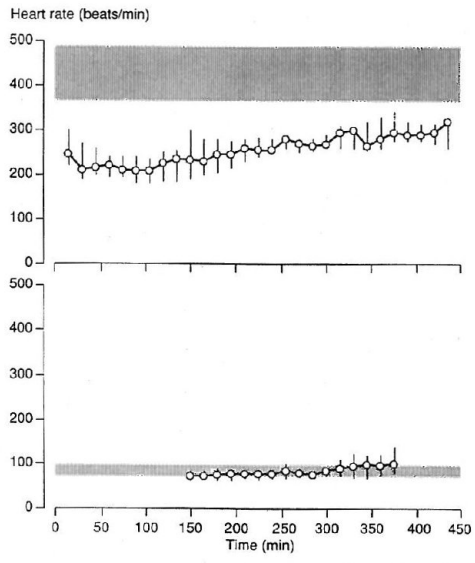

Figure 2. Heart rate in rats (upper graph, n=5) and pigs (lower graph, n=12 (n=6 from 315 min)) while anaesthetized with alpha-chloralose and nitrous oxide. Horizontal bands delineate reference values for conscious animals (80% confidence interval for the median). Values are median and 80% confidence interval. If the 80% confidence interval of values and reference values do not overlap, they are significantly different ($p<0.01$).

ning of alpha-chloralose infusion. A stable pressure of about 125 mmHg, which was significantly higher than the reference values, was obtained in the pigs. In the rats the pressure was significantly lower at the beginning of the experiment and slowly recovering during the experiment. Heart rates during the same period are shown in figure 2. The heart rates in the pig were similar to reference values whereas the heart rates of the rats were significantly lower than reference values throughout the experiment. The rate-pressure product (*Kitamura et al.* 1972) is shown in figure 3. The values for the rat were significantly lower than reference values throughout the experiment whereas the pig had values similar to reference values, except at the end where there was a slight increase. The arterial pCO_2 was similar to reference values for both pigs and rats (figure 4). Figures 5 and 6 shows arterial pH and calculated base excess. The values in the pig did not differ from reference values whereas the rats developed acidosis at the end of the experiment, and the base excess values were significantly lower than reference values from 85 minutes onwards after alpha-chloralose infusion.

Discussion

In both pigs and rats, alpha-chloralose was infused until surgical anaesthesia was obtained. During the experiment heart rate and arterial blood pressure did not increase after applying a pain stimulus. In the pig 180 mg/kg alpha-chloralose was administered to maintain anaesthesia for 375 minutes, while in the rat 288 mg/kg was administered over a period of 435 minutes.

Judging from the mean arterial pressure, which was significantly higher than the reference values in the pig and significantly lower than the reference values in the rat, it can be concluded that the ad-

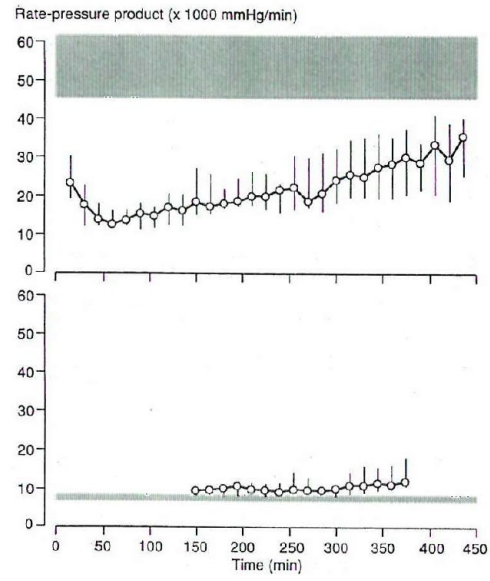

Figure 3. Product of systolic arterial blood pressure and heart rate in rats (upper graph, n=5) and pigs (lower graph, n=12 (n=6 from 315 min)) while anaesthetized with alpha-chloralose and nitrous oxide. Horizontal bands delineate reference values for conscious animals (80% confidence interval for the median). Values are median and 80% confidence interval. If the 80% confidence interval of values and reference values do not overlap, they are significantly different ($p<0.01$).

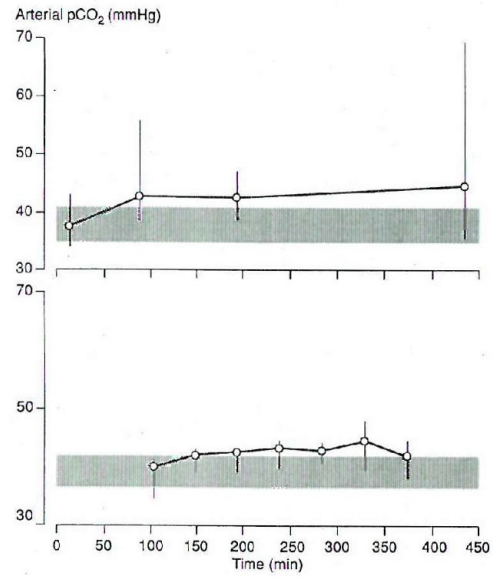

Figure 4. Arterial pCO2 in rats (upper graph, n=5) and pigs (lower graph, n=12 (n=6 from 315 min)) while anaesthetized with alpha-chloralose and nitrous oxide. Horizontal bands delineate reference values for conscious animals (80% confidence interval for the median). Values are median and 80% confidence interval. If the 80% confidence interval of values and reference values do not overlap, they are significantly different ($p<0.01$).

ministered dose of alpha-chloralose to obtain a sufficient level of anaesthesia was low in the pig, while the dose in the rat was high. A similar effect of dosing on blood pressure was seen in the neonatal lamb (*Konduri & Fewell* 1990). In neonatal lambs heart rate and blood pressure were increased by low doses of alpha-chloralose, whereas a high dose caused a decrease in blood pressure. The increase seen following low doses appears to be caused by a two fold increase in plasma norepinephrine concentration and beta-adrenergic receptor stimulation (*Covert et al.* 1992a, 1992b).

The low heart rate and mean arterial pressure in the rat can be explained by the high total dose of alpha-chloralose administered. After the initial average dose of 97 mg/kg, several increments had to be given since tachycardia and a hypertensive reflex occurred after applying a pain stimulus. The total average dose of 288 mg/kg over the experimental period prevented the occurrence of these reflexes.

An impaired peripheral circulation as a result of cardiovascular depression led to a metabolic acidosis in the rat, characterized by a normal pCO_2 and a low arterial pH and calculated base excess. Furthermore, a dilution acidosis may be expected, seen the large volume of infused alpha-chloralose. Dilution of extracellular bicarbonate by infusion of large volumes of isotonic fluid leads to a form of metabolic acidosis described as dilution acidosis (*Wamberg et al.* 1995). Thus, a combined metabolic and dilution acidosis is responsible for the low arterial pH and calculated base excess.

Alpha-chloralose combined with nitrous oxide is a suitable anaesthetic in the pig, showing a normal

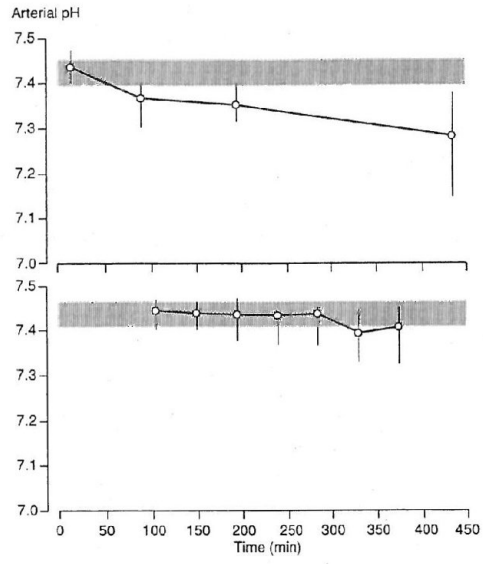

Figure 5. Arterial pH in rats (upper graph, n=5) and pigs (lower graph, n=12 (n=6 from 315 min)) while anaesthetized with alpha-chloralose and nitrous oxide. Horizontal bands delineate reference values for conscious animals (80% confidence interval for the median). Values are median and 80% confidence interval. If the 80% confidence interval of values and reference values do not overlap, they are significantly different ($p<0.01$).

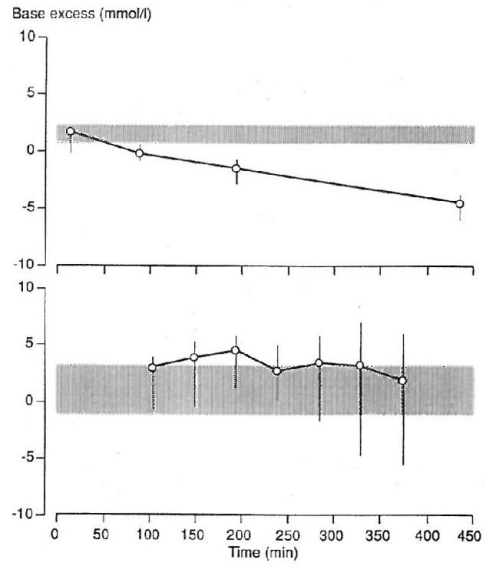

Figure 6. Calculated base excess in rats (upper graph, n=5) and pigs (lower graph, n=12 (n=6 from 315 min)) while anaesthetized with alpha-chloralose and nitrous oxide. Horizontal bands delineate reference values for conscious animals (80% confidence interval for the median). Values are median and 80% confidence interval. If the 80% confidence interval of values and reference values do not overlap, they are significantly different ($p<0.01$).

cardiovascular function and close to normal acid-base status with a satisfactory level of surgical anaesthesia at a low dose. In the rat, alpha-chloralose is an unsuitable anaesthetic since a high dose is necessary to obtain a satisfactory level of surgical anaesthesia, leading to cardiovascular depression and thus to metabolic acidosis. This acidosis is enhanced by dilution acidosis, due to the large volume of infused anaesthetic.

Summary

Alpha-chloralose possesses distinct advantages in certain types of experiments, i.e. in cardiovascular and gastrointestinal research. The compound, however, has limited - if any - analgetic effect, and surgery can only be performed if additional analgesic drugs are administered. In the present study pigs and rats were anaesthetized with a combination of alpha-chloralose and nitrous oxide. A satisfactory surgical anaesthesia could be achieved in pigs using this method, whereas the dose required to obtain surgical anaesthesia in rats caused severe depression of the cardiovascular system. Moreover, a combined metabolic and dilution acidosis was seen in the rat.

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