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What is This?
An effective combination of anaesthetics for 6-h experimentation in the golden Syrian hamster

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Summary

The anaesthetics described for use in hamsters to date are suitable for the performance of short-term experimentation. However, an anaesthetic regimen was required which would provide a stable preparation for 6 h and hence, a suitable combination was developed. In the first set of experiments, the effect of anaesthetics (chloralose, urethane, and pentobarbital) were examined alone and in combination on arterial blood measurements. In the second set of experiments the effect of the combination of anaesthetics on arterial blood measurements and minute ventilation was examined for up to 6 h. Chloralose, urethane and pentobarbital when used alone in the hamster were considered inadequate for our needs. Chloralose did not produce adequate surgical anaesthesia whereas urethane and pentobarbital resulted in marked respiratory depression. Urethane also produced a trend towards metabolic acidosis. In contrast, the combination of agents resulted in surgical anaesthesia and the arterial blood measurements were adequate. Further, the use of the combination of anaesthetics in hamsters resulted in a stable preparation where arterial blood measurements and minute ventilation were maintained in a good range for up to 6 h. The combination of chloralose, urethane and sodium pentobarbital in hamsters should prove useful in long-term non-recovery experimentation which requires early surgical intervention, minimal respiratory depression and an even depth of anaesthesia.

Keywords: Hamsters; Anaesthetics; Pentobarbital; Chloralose; Urethane

The golden Syrian hamster (Mesocricetus auratus) is frequently used for biomedical research. Various modes of inducing anaesthesia in this animal have been described (von Strittmatter, 1972; Green, 1975; Koo et al., 1976; Ferguson, 1979; Green, 1979; Curl & Peters, 1983; Farkas & Roussos, 1984). However, most of these anaesthetic regimens induce a relatively short duration of anaesthesia.

In the performance of long-term physiological studies, an anaesthetic regimen was required which would: (1) induce anaesthesia adequate to allow neck and abdominal surgery; (2) not produce respiratory depression; and (3) allow an even depth of anaesthesia for a 6-h period.

To date, the anaesthetics described for use in hamsters were suitable for the performance of surgery (Koo et al., 1976; Ferguson, 1979; Green, 1979; Curl & Peters, 1983; Farkas & Roussos, 1984). However, prolonged anaesthesia using previously described regimens would require frequent supplemental doses, making the depth of anaesthesia extremely variable. The most commonly employed anaesthetic in the hamster, pentobarbital, produces marked respiratory depression (Borison, 1981; Booth & McDonald, 1982; Seyde et al., 1985) and is difficult to titrate to a standard depth of anaesthesia in other species (Taber & Irwin, 1969).

An effective, long-duration anaesthetic regimen in the hamster was developed using a combination of urethane alpha-chloralose and sodium pentobarbital. This paper describes the effects
of this anaesthetic regimen on anaesthesia time, analgesic response and respiratory control.

**Methods**

**Animals**

Nineteen adult male golden Syrian hamsters (*Mesocricetus auratus*) (Charles River, La Prairie, Quebec) were used in two sets of experiments. They were housed individually in wire mesh cages and maintained on a diet of Purina laboratory chow and water *ad libitum* until the time of the study. They had a mean body weight of 130.0 ± 18.4 g (mean ± SD).

**Experimental design**

In the first set of experiments, 11 animals were divided into 4 groups and anaesthetized with an intraperitoneal injection of one of the following: urethane (n = 3), chloralose (n = 2), pentobarbital (n = 3) or a combination of these agents (n = 3) (see Table 1 for dose rates).

The effects of the anaesthetics on time to anaesthesia, total anaesthesia time, and arterial blood measurements were investigated. Anaesthesia was defined as the state in which the animal was non-responsive to pinching of the paw with surgical forceps and then to surgical incision of the skin.

In a second set of experiments, the combination of anaesthetic agents (urethane, chloralose, pentobarbital) was further examined in 8 animals. Arterial blood gas measurements and minute ventilation were monitored in the animals for up to 6 h. Supplemental doses of urethane and chloralose (13.5 and 1.4 mg/100 g body weight, respectively) were administered every 2 h.

**Animal preparation**

Animals were anaesthetized with an intraperitoneal injection of the anaesthetic agent(s) in a quiet room. Following anaesthesia, they were placed on a heated table with a rectal probe connected to a thermistor. Temperature was maintained at 37 ± 1 °C. Animals were then tracheostomized and allowed to breathe spontaneously through the tracheostomy incision. The left carotid artery was cannulated.

**Sampling of arterial blood**

The left carotid artery was dissected and cannulated using PE10 tubing in order to obtain arterial blood samples. In the first set of experiments, one arterial blood sample was taken one hour after induction of anaesthesia. In the second set of experiments, up to 5 samples of 0.3 ml each were taken during the course of each experiment. The blood volume was replaced with heparinized saline (10 units/ml). The samples were analysed using an ABL 3 acid-base laboratory blood gas analyser (Radiometer, Copenhagen). The analyser was calibrated routinely every two hours. PaO₂ and PaCO₂ were corrected to 37 °C.

**Measurement of minute ventilation and breathing pattern**

Minute ventilation (\(V_E\)) and breathing pattern were determined during spontaneous breathing using a body plethysmograph (Dubois et al., 1956) specially sized for hamsters similar to that described by Koo et al. (1976). The animal was positioned supine in the plethysmograph and the tracheal cannula was attached to a tapered stainless steel tube (14 gauge). Changes in box pressure were used to measure tidal volume. Box pressure was monitored with a ± 2 cm H₂O transducer (Model no. MP45 Validyne Co.,

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**Table 1. Anaesthetic agents used in the study**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dose (mg/100 g body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urethane (50% w/v)</td>
<td>150</td>
</tr>
<tr>
<td>Alpha-chloralose (1% w/v)</td>
<td>8–10</td>
</tr>
<tr>
<td>Pentobarbital (6·5 mg/ml)</td>
<td>5–9</td>
</tr>
<tr>
<td><strong>In combination</strong></td>
<td></td>
</tr>
<tr>
<td>Urethane</td>
<td>38</td>
</tr>
<tr>
<td>Alpha-chloralose</td>
<td>3·8</td>
</tr>
<tr>
<td>Sodium pentobarbital</td>
<td>2·6</td>
</tr>
</tbody>
</table>

*Sigma Chemical Co., St Louis.

* A 1% solution of alpha-chloralose solution was made by heating alpha-chloralose (BDH Chemicals Ltd, Poole, England) and a 10% (w/v) polyethylene glycol (Sigma Chemical Co, MW:6000–7000) to 60 °C. This was administered within 4 h of preparation.

*Somnotol, MTC Pharmaceuticals, Mississauga, Canada.
Northridge, CA). The volume of the plethysmograph was such that tidal breathing produced box pressures which were well within the linear range of sensitivity of the pressure transducer. The box was calibrated at the beginning and the end of the study over the range of 0–10 ml. For measurement of $V_E$ and breathing pattern, a hamster was placed inside the plethysmograph, the plethysmograph was vented several times and the temperature and pressure allowed to equilibrate. The volume signal was recorded (Hewlett Packard multi-channel recorder, Model no. 7758A, Massachusetts) and frequency of breathing ($f_b$), tidal volume ($V_T$), and minute ventilation were determined from the tracing.

**Statistical analysis**

A one-way analysis of variance was performed to determine if there was a difference between the effects of the different anaesthetic agents on arterial blood measurements. Tukey’s test was used to determine which of the groups was significantly different.

**Results**

The results from the first set of experiments examining the effects of the anaesthetic agents alone and in combination are illustrated in Fig. 1 and Table 2. Arterial blood samples were not obtained in the two animals anaesthetized with chloralose because inadequate surgical anaesthesia was obtained such that the tracheostomy and cannulation of the carotid artery could not be performed. Urethane or sodium pentobarbital administered alone in a dose sufficient to produce surgical anaesthesia produced marked respiratory depression as manifested by significant hypercapnia and hypoxemia. In comparison with animals anaesthetized with the combination, those anaesthetized with pentobarbital and urethane had a higher $P_{\text{aco}_2}$ ($P<0.05$) and those anaesthetized with pentobarbital had a

<table>
<thead>
<tr>
<th>Anaesthetic</th>
<th>Time to anaesthesia (min)</th>
<th>Total anaesthesia time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urethane</td>
<td>15</td>
<td>6:00</td>
</tr>
<tr>
<td>Alpha-chloralose</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Pentobarbital</td>
<td>5</td>
<td>0:75</td>
</tr>
<tr>
<td>Urethane, alpha-chloralose and pentobarbital</td>
<td>15</td>
<td>1:5–2:0</td>
</tr>
</tbody>
</table>

**Table 2. Response to anaesthetic agents**

![Fig. 1. The effect of different anaesthetics on arterial blood gas measurements. The raw data for each animal is plotted.](image)

![Fig. 2. The effect of the combination of anaesthetics on arterial blood gas measurements over time. The mean ± SD for each time interval is illustrated.](image)
lower $P_{aO_2}$ ($P<0.05$). The group anaesthetized with urethane had a lower blood pH as compared to the group anaesthetized with the combination ($P<0.05$). In addition to respiratory acidosis, a trend towards metabolic acidosis was associated with the use of urethane. This was demonstrated by a slightly greater base deficit ($-2.2\pm1.9$) in the urethane group than in the other two groups (pentobarbital: $2.7\pm2.5$, combination: $0.4\pm0.5$) ($P=0.066$). The difference did not reach a significant $P$ value because of the small number of animals in each group. In contrast, the $P_{aCO_2}$, $P_{aO_2}$ and pH of the animals anaesthetized with the combination were within the normal range of values for spontaneously breathing hamsters (Lucey et al., 1982). Animals anaesthetized with pentobarbital produced large amounts of saliva requiring frequent suctioning. Excessive salivation was not apparent following administration of the other two agents or combination of anaesthetics.

The effect of the combination of anaesthetic agents on $P_{aCO_2}$, $P_{aO_2}$, pH and ventilation over time is illustrated in Figs 2 and 3 from the second set of experiments. The values were stable over the 6-h period. If there were any changes, there was a trend towards improvement. The increase in $V_E$ was primarily due to an increase in the frequency of breathing with no change in the tidal volume.

**Discussion**

The use of a combination of urethane, alphachloralose and pentobarbital in the hamster resulted in anaesthesia of sufficient depth to permit the performance of surgery and also provided an even depth of anaesthesia without respiratory depression. In contrast, the administration of chloralose, urethane, or pentobarbital alone had limitations. The former did not produce surgical anaesthesia and the latter two agents given alone produced marked respiratory depression.

The use of chloralose (in a dose of 8–10 mg/100 g body weight) as an anaesthetic agent alone resulted in marked skeletal muscle hypertonicity and inadequate surgical anaesthesia in hamsters. Chioralose (like choral hydrate) produces hypnosis and anaesthesia and its metabolism results in the formation of trichloroethanol. The action of
chloralose is typified by functional disruption or dissociation of the central nervous system (CNS) through marked CNS stimulation or induction of a catleptoid state (Booth & McDonald, 1982). Chloralose increases CNS excitability and high doses can result in seizures (Booth & McDonald, 1982) and ultimately respiratory depression (Dripps & Dumke, 1943; Wang & Nims, 1948; Florez & Borison, 1969). Since it only produces stage II anaesthesia (Booth & McDonald, 1982), it is not recommended as an anaesthetic for surgery in other species. Within the limits of the experimental design, this was confirmed in our studies on the hamster since the animals demonstrated marked increased muscle tone and flinching upon touch. Supplemental doses of chloralose only accentuated signs of increased muscle tone and hyperresponsiveness to touch without any apparent deepening of anaesthesia to allow surgical intervention. Because of the hypertonicity of the animal and the inability to do surgery, we were unable to evaluate the respiratory response since both the monitoring of ventilatory levels and arterial blood measurements required surgery.

When urethane alone was administered to the hamster (in a dose of 150 mg/100 g body weight), profound respiratory depression resulted (Fig. 1). In addition, the large decrease in pH was indicative of both a respiratory and metabolic acidosis. The effect of urethane in the hamster in our study was certainly contrary to the results of some investigators (Wang & Nims, 1948; Solman, 1949; Green, 1979) although others (Florez & Borison, 1969) have shown comparable ventilatory depression.

There are several possible causes of the metabolic acidosis found in the hamster in response to urethane. This anaesthetic has been described as causing a decrease in blood pressure (Longnecker & Harris, 1980), dilation of the microvasculature (Landis, 1927; Longnecker & Harris, 1980), leakage of peritoneal fluid (Van der Meer et al., 1975; Severs et al., 1981), and an increase in permeability of the mesenteric microvasculature and other vascular beds (Landis, 1927; Bree & Cohen, 1965) in other species. From our experimental design, we were not able to identify whether or not one of these factors or other causes may have produced the metabolic acidosis.

Urethane had no muscular stimulatory effect on muscle like that observed with chloralose. A single dose of urethane had a long (approximately 6 h) duration of action, similar to the duration of action of chloralose. This is in keeping with the long anaesthesia produced by urethane in other species (Bree & Cohen, 1965; Green, 1979; Booth & McDonald, 1982).

Urethane has been described as being carcinogenic (Green, 1975; Booth & McDonald, 1982) and mutagenic in some species (Green, 1975). Mice, rats and rabbits develop a high incidence of lung tumours when administered urethane. For these reasons, urethane is only suitable for non-survival experiments and laboratory personnel should use appropriate precautions when preparing and using this anaesthetic agent.

An anaesthetic dose of pentobarbital administered to the hamster resulted in marked respiratory depression similar to that described in other species (Borison, 1981; Booth & McDonald, 1982; Seyde et al., 1985). Pentobarbital is a relatively short-acting barbiturate which produces respiratory depression in direct proportion to the amount given. Induction in the hamster was rapid, producing deep anaesthesia of moderate duration (45 min), making it a suitable agent for short surgical procedures. Besides the marked respiratory depression induced by pentobarbital, recovery in the hamster was rapid and unpredictable making this agent unsuitable for experiments requiring long surgical procedures and monitoring of physiological parameters. Excessive salivation (which required repeated suctioning) was another undesirable effect of this anaesthetic.

Because none of the above-mentioned anaesthetics used alone produced adequate results in the hamster, a combination of all three agents was tested. Since both urethane and chloralose do not peak in action until at least 15 min post-injection, pentobarbital was included for a more rapid and smooth induction. Pentobarbital
Six-hour anaesthesia in hamster would be likely to induce the deeper anaesthesia necessary for surgical preparation early in the experimental design. If the animal was anaesthetized with the combination in a quiet environment with minimal handling, loss of consciousness occurred within 5 min and the animal was allowed to rest a further 10 min before being handled.

We were unable to compare the effects of this combination of anaesthetic agents to other studies since we believe the use of this regimen is unique. The effective dose of urethane and chloralose in the hamster was lower than that used in the rat (Hughes et al., 1982; Seyde et al., 1985). The lower dose required in the hamster could be explained by the supplemental use of pentobarbital in our experiments and/or by a different metabolic rate of the anaesthetic agent in hamsters compared to rats. Since we did not investigate the effects of urethane and chloralose in combination it is not possible to compare our results with those of previous studies.

Interestingly, an increase in $V_E$ in the anaesthetized hamster was achieved by an increase in $f_b$ with no change in $V_T$ except at extremely high levels of ventilation.

The alternative use of other anaesthetics such as fentanyl/fluanisone and volatile anaesthetics were considered, however the attributes of the agents chosen appeared superior because of fewer undesirable side effects and ease of administration. The combination of fentanyl/fluanisone has been reported to produce respiratory depression and requires the administration of atropine, the latter of which may further complicate experimental results. The administration of volatile agents require the use of equipment which may complicate measurement of pulmonary function. Further details of advantages and disadvantages of possible alternative anaesthetics are described in the literature (Green, 1975; Green, 1979; Booth & McDonald, 1982).

Although the use of several anaesthetics in combination may be deemed unsuitable for some experimentation because of unpredictable interactions, the use of pentobarbital, urethane and chloralose produced stable reliable anaesthesia in the hamster. Pentobarbital in the combination facilitated a quick, smooth induction and deep anaesthesia to level III so that surgery could be performed. The long duration of action of urethane and chloralose described in other species was also observed in the hamster. Although urethane used alone produced marked respiratory depression, its use in the combination was at a much lower dose and no such effect was observed. The hypertonicity demonstrated by chloralose alone seemed to be countered by the use of the combination by action(s) of either pentobarbital and/or urethane, or again perhaps because of the slightly lower dose.

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