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Comparison of the Depressive Effects of Four Anesthetic Regimens on Ventilatory and Cardiovascular Variables in the Guinea Pig

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***Background and Purpose*: Guinea pigs are one of the most difficult rodents to anesthetize safely, and as a consequence, there is a paucity of reports regarding the effects of anesthesia on their cardiorespiratory variables. We used long-term indwelling cannulas for studying the guinea pig in the conscious state, and subsequently investigated the effects of four types of injectable anesthetic regimens on cardiorespiratory variables.**

***Methods:* Using barometric plethysmography (conscious: long-term cannulated, n = 11; no cannulation, n = 28) or trachea-out plethysmography (anesthetized: n = 7 for each of the four groups), we recorded ventilatory, cardiovascular, metabolic, and arterial gas variables during air breathing and in response to 10 min of hypoxia (8% O_2) and 10 min of hypercapnia (8% CO_2). The four anesthetic regimens tested were: Saffan (infused at 9.75 mg/kg of body weight/h, i.v.); ketamine/xylazine (14.6/3.7 mg/kg/h, i.v.); pentobarbitone (8.3 mg/kg/h, i.v.) plus Innovar Vet (0.15 mg/kg every 1 to 1.5 h, s.c.); or pentobarbitone alone (22 mg/kg/h, i.v.).**

***Results:* The least depressive anesthetic with regard to ventilation ($\dot{V}_E$) was ketamine/xylazine. Air breathing was depressed by only 17% (*cf.* approx 50 to 60% for all other regimes), and the $\dot{V}_E$ responses to hypoxia and hypercapnia were attenuated the least. All anesthetics equally depressed mean arterial blood pressure (from 70 mmHg to 56 mmHg) and ketamine/xylazine was the only anesthetic to reduce heart rate (from 260 beats/min to 198 beats/min).**

***Conclusion:* Although all anesthetics induce cardiorespiratory depression to some extent, the use of ketamine/xylazine is recommended for future use in respiratory studies of the guinea pig where anesthesia cannot be avoided.**

Guinea pigs are common laboratory rodents used for scientific research. The invasive surgical procedures that are often involved necessitate the use of anesthesia. However, guinea pigs are among the most difficult rodents in which to achieve safe and effective anesthesia (13).

Although the guinea pig has been the focus of numerous anesthesia trials, many investigators have principally investigated the pharmacodynamic properties, not cardiorespiratory consequences of anesthesia in guinea pigs; consequently, there are few data regarding the effects of anesthesia on cardiorespiratory variables in the guinea pig. Brown and co-workers (5) measured respiratory rate, but only by visually counting the number of breaths per minute. Other investigators have reported the "absence of respiratory depression" without providing evidentiary data (12, 29). Although Barzago and co-workers (3) provided data for tidal volume (V_T) and respiratory frequency (f) for ketamine/xylazine anesthetized guinea pigs, data for conscious animals were not provided to determine accurately the magnitude of respiratory depression. Our laboratory workers reported that the baseline minute volume of ventilation (expired) ($\dot{V}_E$) of pentobarbitone-anesthetized guinea pigs (28) was approximately 30% lower than that for conscious guinea pigs (9).

Furthermore, to our knowledge, there are no reports of the effects of anesthesia on the $\dot{V}_E$ responses of the guinea pig to hypoxia or hypercapnia. Although pentobarbitone anesthesia depresses baseline $\dot{V}_E$, we have reported that the magnitude of the $\dot{V}_E$ response to 8% O_2 (30% increase [28]) appeared similar to that expected for a conscious guinea pig (26% increase [9]). Furthermore, the $\dot{V}_E$ response to 10% O_2 is negligible for conscious (1) and anesthetized (28) guinea pigs. The magnitude of the $\dot{V}_E$ response to hypercapnia, like hypoxia, appears to be unaltered by pentobarbitone anesthesia. For example, the $\dot{V}_E$ response to 6% CO_2 is similar for conscious (200% increase [1]) and anesthetized (217% increase [28]) guinea pigs.

In the study reported here, we aimed to find an injectable anesthetic regimen that would cause the least degree of $\dot{V}_E$ and cardiovascular depression—for future use in studies of the guinea pig where anesthesia is necessary. Since the focus of our research is to test a range of inhaled gases low in O_2 or high in CO_2, we avoided use of inhalation anesthetics as this would complicate administration of such test gases. On the basis of the limited available information in literature, we selected for testing four anesthetic regimens that were ranked from: likely to be least depressive to likely to be most depressive. The anesthetics

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were: Saffan, ketamine/xylazine combination, pentobarbitone sodium and fentanyl-droperidol combination (Innovar-Vet), and pentobarbitone sodium alone.

Materials and Methods

Animals. Experiments were conducted on 39 female Hartley guinea pigs (approx. 100 days old; body weight approx. 615 g) obtained from the University of Otago's animal breeding unit. According to the unit's microbiology laboratory, the guinea pigs were free of pneumonia virus of mice, reovius, Sendai virus, *Encephalitozoon cuniculi*, *Bordetella bronchiseptica*, *Streptococcus pneumoniae*, *S. zooepidemicus*, *Klebsiella* sp., *Staphylococcus aureus*, *Salmonella* sp., ectoparasites, and *Eimeria* sp., but were test positive for *Balantidium coli* and trichomonads (endoparasites). Eleven guinea pigs were studied only in the conscious state, before and after indwelling arterial and venous catheters were implanted. Twenty-eight guinea pigs were studied in the conscious state (uninstrumented) and after being anesthetized using one of four anesthetic regimens (i.e., four groups of seven). Guinea pig housing was on a 12-h light/dark cycle at 20 ± 2°C, with relative humidity between 35 and 50%. Guinea pigs were provided food (food pellets, hay, and cabbage) and water ad libitum. All experiments were approved by the local animal ethics committee.

Experimental protocols. All 39 guinea pigs were subjected to the experimental tests in the conscious state. However, each guinea pig had to first be trained to sit restfully in the plethysmograph to avoid stress or anxiety, which would influence the results (7). Training was achieved by exposing each guinea pig to the experimental protocol once a day for three consecutive days. Once trained, the conscious guinea pig was subjected to the experimental tests.

On the day of the experiment, each guinea pig initially was allowed 30 min to settle in the plethysmograph. Another 30 min of air breathing was recorded before guinea pigs were randomly exposed to hypoxia (8% O_2 in N_2) for 10 min and hypercapnia (8% CO_2, 21% O_2 in N_2) for 10 min. Each test gas was followed by 15 min of air breathing for the recovery period. The duration of the experimental protocol was no longer than two hours.

On the day after the experiment, guinea pigs were subjected again to the experimental tests, but in the anesthetized state (n = 28), or they underwent aseptic surgery to insert arterial and venous cannulas (n = 11). On recovery from surgery (three to four days) long-term cannulated guinea pigs were retrained for one day, then were subjected to the experiment tests, again in the conscious state, on the subsequent day. All guinea pigs were euthanized by administration of an anesthetic overdose at the end of the experiment.

Surgical preparation. For long-term cannulation each guinea pig was anesthetized with N_2O (1 L/min)/O_2 (2 L/min) and halothane (2 to 4%). Rectal temperature was maintained at 39°C, using a rectal thermistor coupled with a thermostatically controlled heating pad. Using standard aseptic techniques, skin incisions were made distally down the nape of the neck and distally down the ventral surface of the groin. The arterial and venous cannulas were filled with sterile heparinized saline (40 U/ml) and tunneled subcutaneously through both incision sites. The rostral ends of the cannulas were passed through a stainless steel disk and spring coil.

Each cannula consisted of a 50-cm length of polyethylene tubing (0.80-mm ID, 1.20-mm OD) 'welded' to another 6.5 cm of polyethylene tubing (0.50-mm ID, 0.8-mm OD). The 6.5-cm part of the cannula was inserted into the femoral artery or vein so that the tip of the cannula was positioned in the abdominal aorta. The cannulas were sutured to the adductor magnus muscle to prevent dislodgment, and the groin incision was closed. The stainless steel disk was sutured and anchored to the muscle on the back of the neck; then, the nape of the neck was closed with sutures.

Figure 1. Photograph of a conscious, long term-cannulated guinea pig in its modified housing, using a gimbal device to support the cannulae. The spring coil encases and protects the cannulae, which exit from the nape of the neck. The spring coil is attached to a roller on the crane-like arm that allows the instrumented guinea pig full range of movement around the housing.

Immediately after surgery, all animals received an injection (i.m.) of Temgesic (Buprenorphine [0.05 mg/kg of body weight], Reckitt & Colman Products Ltd., Dansom Lane, Hull, UK) for analgesia, then were placed in a modified cage that incorporated a 'gimbal device' (Fig. 1), and were continuously infused (i.v.) with saline for five to six hours at a rate of five milliliters per hour. The freely moving gimbal device permitted the animal to move and rotate freely within the cage without the cannulas becoming tangled.

Animals required three to four days to fully recover from surgery. Recovery was based on: body weight having at least returned to the pre-surgery weight (or weight was increasing at a rate of four to five grams per day, which is the normal weight gain for an 80- to 120-day-old guinea pig), and the food and water intake being normal for a 600-g guinea pig (personal communication with Dr. John Schofield, Director of Animal Welfare, University of Otago). Guinea pigs were reluctant to drink after surgery. Therefore, saline was infused (three milliliters per hour, i.v.) for eight hours each day until water consumption had returned to normal (two to three days). To maintain cannula patency, they were flushed daily with freshly prepared heparinized saline.

For experiments on anesthetized animals, guinea pigs were initially anesthetized with the N_2O/O_2/halothane anesthetic mixture. The femoral vein, femoral artery, and trachea were cannulated in that order, although aseptic surgery and subcutaneous tunneling were not required. Prior to tracheotomy, the N_2O/O_2/halothane anesthetic mixture was stopped and intravenous administration of

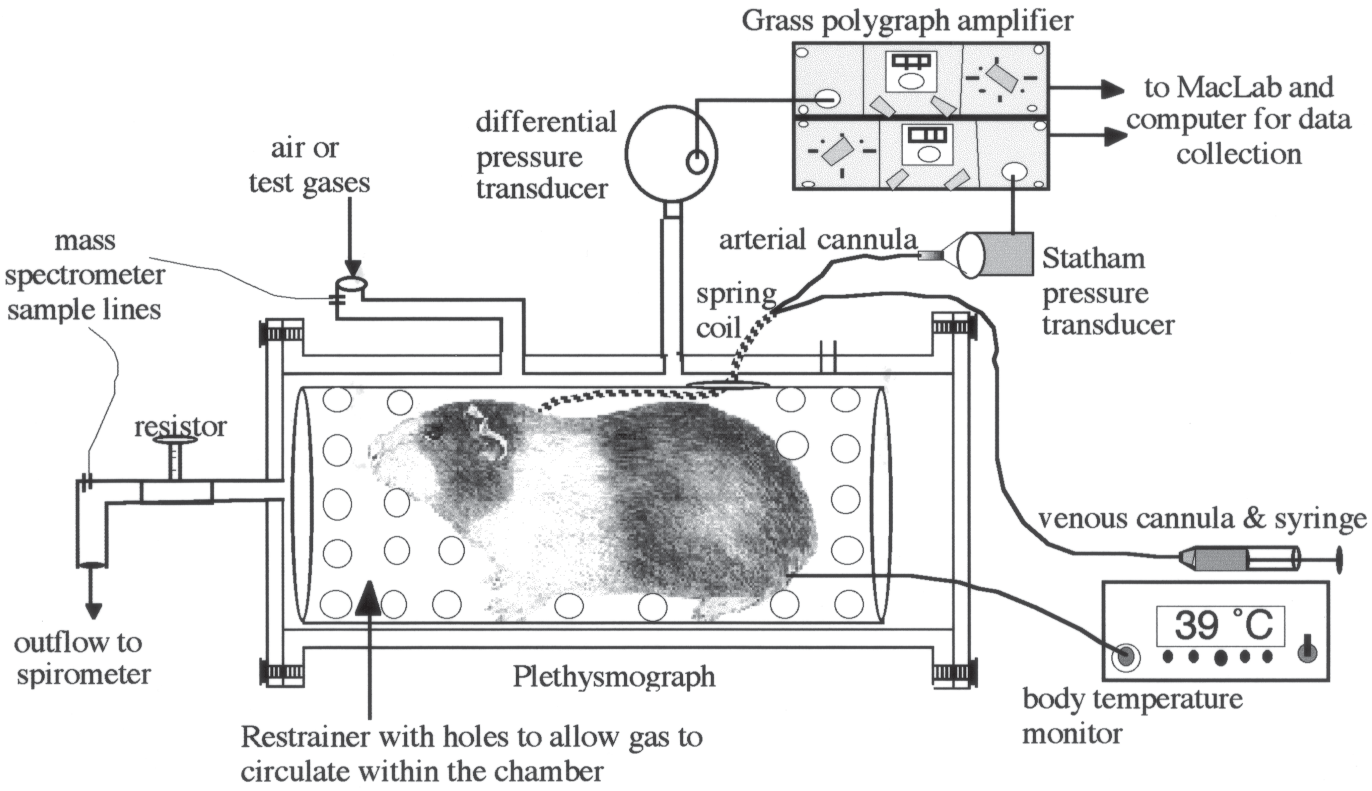

Figure 2. Schematic diagram of the equipment and experimental set-up used for the whole-body plethysmography procedure.

the selected anesthetic was started. It was important to wait sufficient time (approx. 30 sec) for excess halothane to be eliminated before beginning intravenous administration of anesthetics; otherwise, the sum of the two anesthetics could result in respiratory depression and death. Therefore, anesthetic was injected slowly (over a period of one to four minutes).

Intravenous anesthesia. The initial concentration selected for each anesthetic was based on that reported in literature. Using the first four guinea pigs for each regimen, two hours was spent prior to the experimental protocol determining the ideal infusion rate for each anesthetic by periodically administering anesthetic so that a slight limb withdrawal reflex remained in response to a firm pinch of a hind foot pad. At this depth of anesthesia, the corneal reflex was abolished and breathing and blood pressure were stable (i.e., no erratic fluctuations). Therefore, the limb withdrawal reflex was the test selected for assessing anesthesia depth during the experimental protocol. We consider anesthesia too deep if the limb withdrawal reflex to a firm pinch of a hind foot pad is completely abolished, because this depth of anesthesia always results in severe respiratory depression and visual signs of cyanosis. Once the amount of anesthetic required per kilogram per hour was evaluated, the intravenous line was connected to an infusion pump. For each regimen, the intravenous cannula in the final three guinea pigs was connected directly to the infusion pump immediately after surgery and the infusion rate was set accordingly. Adjustments in infusion rate were easily performed, if required, to maintain a satisfactory depth of anesthesia.

When Saffan (alphaxalone-alphadalone, Pitman-Moore, Lower Hutt, New Zealand) was used, an initial intravenously administered bolus dose of 4.5 mg/kg was slowly injected (over 30 sec) after the N_2O/O_2/halothane apparatus had been removed. During experiments, the infusion rate of Saffan to maintain adequate anesthesia was 9.75 mg/kg/h.

Ketamine HCl and xylazine (2%) were used in combination. The two anesthetics were combined (ketamine: 20 mg/kg; xylazine: 5 mg/kg) to give a combination ratio of 1:1.25. An initial dose of 5.5 mg of ketamine/kg/1.4 mg of xylazine/kg (i.e., 5.5/1.4 mg/kg) was injected over a 30-sec period after the N_2O/O_2/halothane mixture was removed. During experiments, the infusion rate of ketamine/xylazine was 14.6/3.7 mg/kg/h.

Pentobarbitone sodium was used alone or in conjunction with a fentanyl/droperidol mixture (also known as Innovar Vet, Pitman-Moore, Ill.). Various routes were used for administering pentobarbitone (i.v.) and Innovar Vet (s.c.), since they form a precipitate when combined. An initial dose of 10 mg of pentobarbitone/kg was slowly injected (i.v.) over a two-minute period after the N_2O/O_2/halothane mixture was removed. During experiments, pentobarbitone was infused at a rate of 22 mg/kg/h if used alone, or 8.3 mg/kg/h when used in conjunction with Innovar Vet, which was administered (s.c.) intermittently throughout the experiment (0.15 mg/kg, every 1 to 1.5 h).

Experimental set-up. The barometric (whole-body) plethysmograph was used when the guinea pig was studied in the conscious state, and the trachea-out technique was used when the animal was studied in the anesthetized state.

Barometric, or whole-body plethysmography that incorporated the flow-through approach was used to continuously measure V_T (Fig. 2). Use of the 'flow-through' technique has been reported in the literature (6, 15), and is a modification of the closed system technique that was first described by Drorbaugh and Fenn (10). For a review regarding the principle and accuracy

of measurement, the reader is referred to publications by Mortola and Frappell (24) and Enhorning and co-workers (11).

Changes in chamber pressure associated with inspiration and expiration were continuously measured on top of a continuous gas-flow background through the plethysmograph. This was achieved by incorporating a resistance to airflow exiting the circuit. Breathing-associated changes in pressure are accurately proportional to V_T when the time required for a square wave increase in pressure to return to baseline (termed 'leak time') is greater than two seconds. In this study, the maximal inspiratory and expiratory times (T_I, 0.34 sec and T_E, 0.38 sec) were always shorter than the leak time.

Gases flowing through the circuit were humidified (100% saturation) and heated to ensure that the chamber was within the thermoneutral zone for guinea pigs, which is 28.5 to 29.8°C (2). It is essential to maintain a chamber temperature that is constant and within the animal's thermoneutral zone to accurately measure V_T (24) and baseline oxygen consumption ($\dot{V}O_2$) (7). Chamber temperature and body temperature were continuously measured (to one decimal place) by use of electronic thermometers. Body temperature was approximately 39°C (range, 38.8 to 39.1°C).

Long-term-cannulated guinea pigs were prevented from rotating within the plethysmograph, which could entangle the cannulas, by using an adjustable 'restrainer'. The arterial and venous cannulas were exteriorized through a porthole in the top of the plethysmograph and were secured with plasticine to ensure an airtight seal.

Anesthetized guinea pigs. The trachea-out plethysmography technique was used. After surgery, the animal was placed in the supine position inside a cylindrical perspex plethysmograph. The tracheal, venous, and arterial cannulas were exteriorized through the wall of the plethysmograph, so that the animal breathed air from outside of the chamber. Air and test gas mixtures were delivered (two liters per minute) from four gas rotameters (air, O_2, CO_2, and N_2) through a single polyethylene tubing across the exteriorized tracheal cannula.

Since the animal breathed air from outside the plethysmograph, pressure fluctuations within the airtight chamber were produced by inspiration and expiration and these fluctuations were directly proportional to V_T.

Although different techniques were used to measure V_T in conscious and anesthetized guinea pigs, results of preliminary experiments in our laboratory have indicated that, when a guinea pig is studied, first in the conscious state (i.e., whole-body plethysmography), then immediately in the anesthetized state (i.e., trachea-out plethysmography), the value for V_T is identical (8).

Data acquisition. Irrespective of whether the whole-body or trachea-out plethysmograph was used, pressure changes in the chamber were detected by use of a volumetric pressure transducer (Grass PT5A, Grass Instrument Company, Quincy, Mass. [at time of purchase]–company now Astro-Med. Inc. Grass Product–Group, West Warwick, Rhode Island) attached to the plethysmograph. Arterial blood pressure (ABP) was continuously measured by use of a Statham pressure transducer (Model P23AC, Statham Medical Instrument Inc., Hato Rey, Puerto Rico). The signals for V_T and ABP were amplified (Grass Polygraph D.C. Driver Amplifiers Model 7DAF, Grass Instrument Company, Quincy, Mass. [at time of purchase]—company now Astro-Med. Inc. Grass Product— Group, West Warwick, Rhode Island), sampled at 200 Hz using an eight-channel MacLab/8s interface hardware system (AD Instruments, Pty Ltd., Castle Hill, NSW, Australia), and were recorded on a Macintosh Power PC, using the program 'Chart' (3.5.7/s). Respiratory frequency and heart rate (HR) were derived from the V_T and ABP signals, respectively, and were displayed on separate channels. The $\dot{V}_E$ value was calculated off line as the product of V_T and frequency, and was normalized to 100 g of body weight (BTPS).

Arterial blood samples (0.15 ml) were taken at regular intervals during the experimental protocol and were analyzed for pHa, PaO_2, and $PaCO_2$ (at 39°C), using an ABL 50 blood gas analyzer (Radiometer, Copenhagen, Denmark), then were analyzed for lactate concentration, using a lactate analyzer (YSI model 1500 sport Lactate Analyser, Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio). The total volume of blood extracted was 0.45 ml, which was replaced by a similar volume of saline.

The $\dot{V}O_2$ and carbon dioxide production ($\dot{V}CO_2$) were calculated as the difference between the gas fraction of the inlet and outlet gases multiplied by the flow-rate. The Haldane correction factor was incorporated into the calculations to account for the fact that, normally, the inlet flow rate is greater than the outlet flow rate (30). Gas fractions were measured by use of a mass spectrometer (MGA-1100 Medical Gas Analyser, Marquette Electronics, Milwaukee, Wis. [at time of purchase] company now General Electric Medical Systems, Waukesha, Wis.). The difference between the fractions of inlet and outlet oxygen ranged from 0.0035 to 0.0055. A flow rate of 2 L/min, which was precisely measured using a spirometer (Palmer Ltd., London, England), was set, and $\dot{V}O_2$ and $\dot{V}CO_2$ were expressed at standard temperature and pressure (STPD), and were normalized to 100 g of body weight (i.e., ml/min/100 g STPD).

Data analysis. Ventilatory and cardiovascular variables (the latter where applicable) were continuously recorded during the experimental protocol. During the 30 min of air breathing, there was no significant change in any of the variables; thus, the average air value for each guinea pig was collated. Data were also analyzed during the ninth minute of exposure to hypoxia and hypercapnia. The $\dot{V}O_2$ was measured at 10-min intervals during the 30 min of air breathing, and an average was obtained. The $\dot{V}O_2$ was also measured between the six- and eight-minute interval of each test gas. For the cannulated guinea pigs (conscious and anesthetized), a blood sample was extracted for measurement of PaO_2, $PaCO_2$, pHa, and arterial lactate values during the fifteenth minute of air breathing, and during the seventh minute of hypoxia and hypercapnia.

Statistical analysis. All statistical analyses were completed, using Statview (v5.01). All results are presented as mean ± SEM. Two-way analysis of variance (ANOVA) was used to test significance for: changes in ventilation during 30 min of air breathing for each group of guinea pigs; separate for conscious and anesthetized states (with repeated measures); changes in ventilation in response to hypoxic and hypercapnic test gases for each group of guinea pigs; conscious and anesthetized states (with repeated measures); and interaction between the ventilatory responses for conscious, compared with anesthetized groups of guinea pigs (factorial).

One-way ANOVA (factorial) was used to test significance for: air, hypoxia, or hypercapnia values, separately, among all groups of guinea pigs; differences in the ventilatory responsiveness to

Table 1. Ventilatory and cardiovascular variables of conscious, long-term cannulated, guinea pigs (n = 11), and four groups of anesthetized guinea pigs (n = 7/group) during air breathing

Group	$\dot{V}_E$ (ml/min/100 g)	V_T (ml/100 g)	f (/min)	$\dot{V}O_2$ (ml/min/100 g)	$\dot{V}CO_2$ (ml/min/100 g)	R breaths/min)	MABP (mmHg)	HR (/min)	PaO_2 (mmHg)	$PaCO_2$ (mmHg)	pH	Lactate (mmol/L)
Conscious	52	0.65	81	1.36	1.14	0.85	70	267	98	33	7.415	0.65
	± 1	± 0.02	± 2	± 0.05	± 0.03	± 0.02	± 2	± 7	± 2	± 1	± 0.013	± 0.05
Saffan	21†	0.48†	44†	0.91†	0.84†	0.92	59†	272	77†	43†*	7.433	0.66
	± 1	± 0.04	± 4	± 0.03	± 0.03	± 0.02	± 1	± 9	± 3	± 1	± 0.013	± 0.06
Ketamine/	43	0.56	77†	1.01†	0.89†	0.88	54†	198‡	79†	38†	7.392	0.75
xylazine	± 2	± 0.02	± 3	± 0.07	± 0.04	± 0.04	± 5	± 7	± 2	± 1	± 0.005	± 0.07
Pentobarbitone/	25†	0.65	38†	0.89†	0.77†	0.86	53†	259	78†	39†	7.446	0.71
Innovar Vet	± 1	± 0.07	± 4	± 0.03	± 0.02	± 0.02	± 2	± 6	± 1	± 1	± 0.009	± 0.05
Pentobarbitone	26†	0.67	39†	0.97†	0.85†	0.88	56†	284	79†	37†	7.423	0.73
	± 1	± 0.05	± 2	± 0.03	± 0.04	± 0.02	± 2	± 7	± 1	± 1	± 0.020	± 0.05

*The $PaCO_2$ of Saffan-anesthetized guinea pigs was significantly ($P < 0.05$) higher, compared with values for all other groups. †Significantly different from value for the the conscious group ($P < 0.05$). ‡Heart rate (HR) of ketamine/xylazine-anesthetized guinea pigs was significantly ($P < 0.05$) lower, compared with values for all other groups. Data are expressed as mean ± SEM.
$\dot{V}_E$ = minute volume of ventilation (expired); V_T = tidal volume; f = respiratory frequency; $\dot{V}O_2$ = O_2 consumption; $\dot{V}CO_2$ = CO_2 production; R = respiratory exchange ratio; MABP = mean arterial blood pressure.

hypercapnia (linear regression lines for $\dot{V}_E$ versus $PaCO_2$) for conscious, long-term cannulated versus anesthetized groups of guinea pigs. Post-hoc analyses were done for the aforementioned comparisons, using the paired, or unpaired *t*-test, with the Bonferroni/Dunn correction incorporated for multiple comparisons. A value of $P = 0.05$ was predetermined as the level of significance for all statistical analysis.

Results

After surgery, two hours was required to determine the correct infusion rate of anesthetic, and to allow sufficient time for the guinea pig's condition to stabilize. Assessment of anesthesia was achieved, using the limb withdrawal reflex. During this two-hour period, specific observations were made for each group of anesthetized guinea pigs.

Saffan. Guinea pigs were sensitive to the transition from halothane to Saffan (i.v.). Often respiratory arrest would ensue, although it was short lived and animals were easily resuscitated by taking two to three breaths from a mechanical ventilator. Saffan provided adequate anesthesia, but only when the required dose was near the lethal dose. Additionally, the therapeutic index range for Saffan was narrow (i.e., the correct infusion rate was often difficult to determine; anesthesia fluctuated between light and deep levels).

Ketamine/xylazine. This combination was generally good so that surgical anesthesia was attained at low doses. The transition to the anesthetic combination (i.v.) was consistently smooth. Bolus injections of anesthetic (0.1 ml) were sometimes used during the initial one-hour period. These bolus injections induced transient increases of 20 to 30 mmHg in mean ABP (MABP). During experimentation, the animals did not experience adverse side effects to this anesthetic regimen. The wide therapeutic range of ketamine/xylazine ensured that the correct infusion rate was evaluated quickly (< 40 min).

Pentobarbitone-Innovar Vet combination. Finding the right ratio for pentobarbitone (i.v.) and Innovar Vet (s.c.) was difficult. The fentanyl component of Innovar Vet, although described as a good analgesic, was potent in depressing respiration within five minutes of each injection, and its effects lasted for approximately 10 to 15 min. Consequently, breathing fluctuated with doses of Innovar Vet. Complications were further added by constantly adjusting the infusion of pentobarbitone to compensate for the effects of Innovar Vet.

Pentobarbitone. The dose required to provide sufficient anesthesia was near the lethal dose. The correct infusion rate with which to achieve a steady breathing pattern was difficult to determine, often starting with a high infusion rate but then gradually decreasing the infusion rate over a three- to four-hour period. All guinea pigs tested using pentobarbitone had excess tracheal secretions; such was not associated with all other anesthetic regimens tested. This procedure often obstructed breathing, and hence delayed the experiment until the secretions were cleared by suction. Use of atropine (60 µg/kg, s.c.) in three guinea pigs did not inhibit tracheal secretion or affect any of the measured cardiorespiratory variables.

Cardiorespiratory measurements. Data were collected from five groups of conscious guinea pigs: the conscious, long-term-cannulated group, and the four groups that were subsequently studied in the anesthetized state. All ventilatory and metabolic steady-state data acquired during air breathing and in response to hypoxia (8% O_2) and hypercapnia (8% CO_2) were similar for all conscious groups. On the basis of these results, it is likely that the MABP, HR, and arterial blood variables reported for the long-term-cannulated guinea pig are representative of a conscious uncannulated guinea pig. Additionally, since all ventilatory data were similar for all conscious groups, only the conscious data from the long-term-cannulated guinea pig were used for comparing with data for the anesthetized groups (since MABP, HR and arterial gas values can be compared).

Air breathing. In general, anesthesia caused respiratory and metabolic depression (Table 1). Ketamine/xylazine caused the least degree of $\dot{V}_E$ depression (17%), compared with that for all other regimens (50 to 60%). Anesthesia predominantly depressed respiratory frequency, with little or no effect on V_T (except for a 26% decrease in V_T for the Saffan group). The depression in $\dot{V}O_2$ was similar to the magnitude of ventilatory depression in the ketamine/xylazine group whereas in all other groups the depression in $\dot{V}O_2$ was less than the depression in $\dot{V}_E$. The PaO_2 and $PaCO_2$ in guinea pigs of the conscious group were higher and lower, respectively, compared with those of guinea pigs of all anesthetized groups, whereas the arterial lactate and pH values were not affected by anesthesia (Table 2). The MABP for all anesthetized groups was statistically similar, but gener-

Table 2. Arterial gas, lactate, and cardiovascular variables of conscious and anesthetized guinea pigs during air breathing and in response to 10 min of hypoxia (8% O_2) and 10 min of hypercapnia (8% CO_2)

Group	Test	PaO_2 (mmHg)	$PaCO_2$ (mmHg)	pHa	Lactate (mmol/L)	MABP (mmHg)	HR (/min)
	Air	98 ± 2	33 ± 1	7.415 ± 0.013	0.65 ± 0.05	70 ± 2	267 ± 7
Conscious (n = 11)	8% O_2	23* ± 1	28* ± 1	7.486* ± 0.012	1.43* ± 0.20	52* ± 3	259 ± 9
	8% CO_2	133* ± 2	53* ± 1	7.275* ± 0.016	0.57 ± 0.05	68* ± 2	284 ± 5
	Air	77† ± 3	43†§ ± 1	7.433 ± 0.013	0.66 ± 0.06	59† ± 1	272 ± 9
Saffan (n = 7)	8% O_2	16*† ± 1	37*† ± 1	7.470 ± 0.015	1.47* ± 0.17	41*† ± 5	262 ± 15
	8% CO_2	127* ± 2	57*† ± 1	7.303* ± 0.008	0.77† ± 0.08	55 ± 3	265 ± 9
	Air	79† ± 2	38† ± 1	7.392 ± 0.005	0.75 ± 0.07	54 † ± 5	198†‡ ± 7
Ketamine/ xylazine (n = 7)	8% O_2	23*‡ ± 1	35† ± 2	7.422 ± 0.019	1.53* ± 0.08	27 *‡† ± 1	189†‡ ± 10
	8% CO_2	133* ± 5	52* ± 2	7.318* ± 0.019	0.78† ± 0.05	47†‡ ± 7	193†‡ ± 9
	Air	78† ± 1	39† ± 1	7.446 ± 0.009	0.71 ± 0.05	53† ± 2	259 ± 6
Pentobarbitone/ Innovar Vet (n = 7)	8% O_2	16*† ± 1	33*† ± 1	7.509* ± 0.023	1.41* ± 0.11	36*† ± 2	245 ± 14
	8% CO_2	128* ± 2	54* ± 1	7.319* ± 0.014	0.75† ± 0.07	58* ± 2	257 ± 8
	Air	79† ± 1	37 † ± 1	7.423 ± 0.020	0.73 ± 0.05	56 † ± 2	284 ± 7
Pentobarbitone (n = 7)	8% O_2	17*† ± 0	33*† ± 1	7.430 ± 0.018	1.49* ± 0.08	38*† ± 1	262 ± 13
	8% CO_2	126* ± 3	53* ± 1	7.331* ± 0.037	0.68 ± 0.06	60 ± 2	274 ± 8

*Significantly different from air values. †Significantly different from value for the conscious group ($P < 0.05$). ‡Ketamine/xylazine-anesthetized guinea pigs had significantly ($P < 0.05$) lower HR and MABP, and significantly ($P < 0.05$) higher baseline PaO_2, compared with all other groups. §Baseline $PaCO_2$ of Saffan-anesthetized guinea pigs was significantly ($P < 0.05$) higher than that for all other groups.
See Table 1 for key.

ally 20% lower than that obtained for the conscious, long-term cannulated group. The HR for the ketamine/xylazine group (198 beats/min [bpm]) was lower than that for all other anesthetized groups: HR values were similar to those for the conscious group.

Hypoxia. In the conscious guinea pig, hypoxia (8% O_2) caused a significant 44% increase in $\dot{V}_E$ that was caused by a 26% increase in V_T and a 15% increase in frequency (Fig. 3). Hypoxia decreased $\dot{V}O_2$ by 29% and decreased MABP by 26% (Table 2). Body temperature did not change during hypoxia.

In general, anesthesia was detrimental to the magnitude of the hypoxic ventilatory response. The ketamine/xylazine group had the largest $\dot{V}_E$ response to hypoxia (27% increase), and it was the only group that had an increase in V_T (14% increase). All other groups responded solely with an increase in respiratory frequency. Hypoxia depressed $\dot{V}O_2$ in the anesthetized groups by a magnitude similar to that of the conscious guinea pig, ranging from an 8 to 35% decrease (Fig. 3). Body temperature was kept constant in anesthetized guinea pigs. The significant hypoxia-induced decrease in MABP in the conscious guinea pig (26% decrease) was accentuated only by ketamine/xylazine anesthesia (50% decrease). Heart rate and pHa were not altered during hypoxia in any of the anesthetic groups (Table 2).

Hypercapnia. In conscious guinea pigs, hypercapnia induced a 350% increase in $\dot{V}_E$ that was caused by a 146% increase in V_T and an 80% increase in frequency (Fig. 4). The ventilatory responsiveness to changes in $PaCO_2$ of conscious guinea pigs, (i.e., the slope of the $\dot{V}_E$ versus $PaCO_2$ line) was 9.055 ml/min/100 g/mmHg (Fig. 5). Hypercapnia did not significantly alter $\dot{V}O_2$, MABP, HR or arterial lactate values, but it decreased pHa (pHa change of 0.14) and increased PaO_2 by 36% (Table 2). Body temperature did not change during hypercapnia.

The magnitude of increase for $\dot{V}_E$, V_T, and frequency was statistically similar for all anesthetized groups of guinea pigs. Although anesthesia did not alter the 'magnitude' of the ventilatory response to hypercapnia (i.e., percentage increase), the $\dot{V}_E$ sensitivity to hypercapnia (slope for $\dot{V}_E$ versus $PaCO_2$) was significantly attenuated by all anesthetic regimens, but least for the ketamine/xylazine group (7.071 ml/min/100 g/mmHg) (Fig. 5). Generally, hypercapnia had little or no effect on $\dot{V}O_2$, MABP, or HR in anesthetized guinea pigs (Table 2).

Discussion

Invasive physiologic research ultimately requires anesthesia. Unfortunately, the guinea pig has been described as one of (if not) the most difficult animal to safely anesthetize (5, 14, 17, 22). The available literature investigating anesthetic-induced respiratory depression in the guinea pig is scarce. In the study reported here, we used conscious, long term-cannulated guinea pigs for measuring ventilatory as well as cardiovascular and arterial gas variables, and then investigated the effects of four injectable anesthetic regimens on these variables.

The term 'anesthesia' refers to the absence of all sensation. The term 'analgesia' refers only to the absence of pain perception. When a surgical plane of anesthesia has been attained, all sensation, including pain, has been suppressed. However, use of one anesthetic agent alone often requires a high dose to elimi-

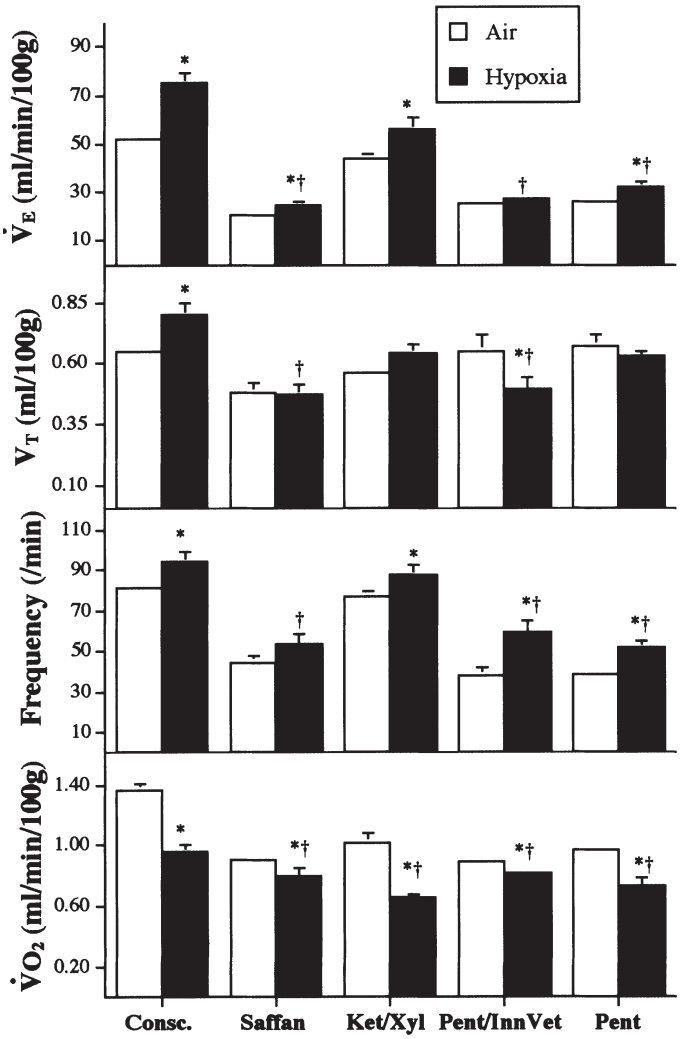

Figure 3. Ventilatory and metabolic responses to hypoxia (8% O_2) of one conscious (cannulated) group (n = 11) and four anesthetized groups of guinea pigs (n = 7/group). *Significantly different from baseline (i.e., air) values. †Significantly different from the value for the conscious, long term-cannulated group (hypoxia values only, $P < 0.05$).

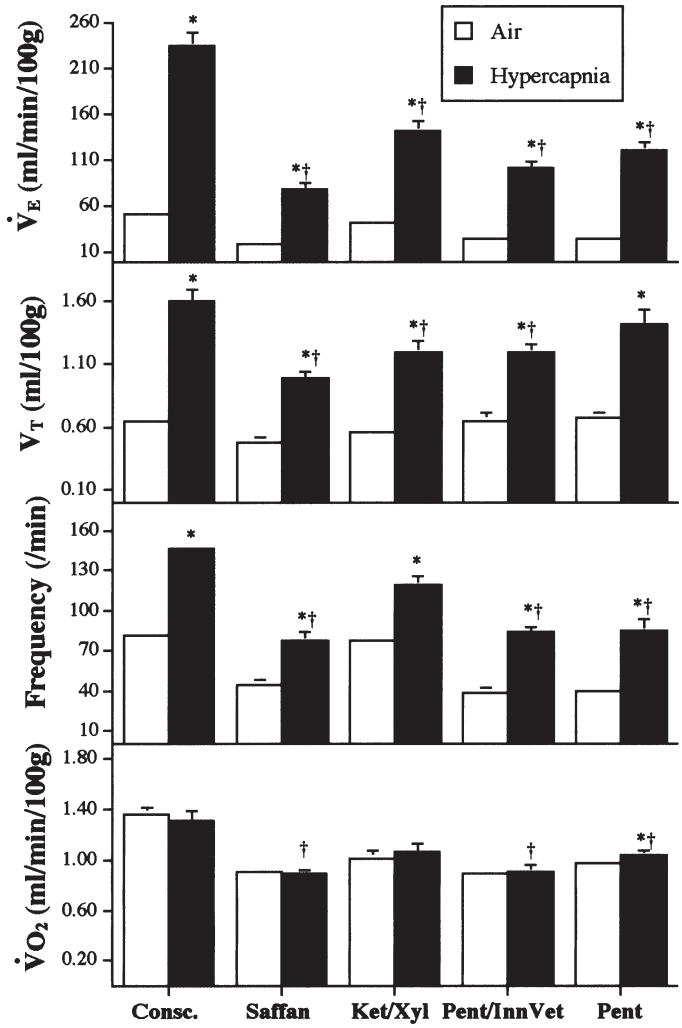

Figure 4. Ventilatory and metabolic responses to hypercapnia (8% CO_2) of one conscious (cannulated) group (n = 11/group) and four anesthetized groups of guinea pigs (n = 7/group). *Significantly different from baseline (i.e., air) values. †Significantly different from the value for the conscious, long term-cannulated group (hypercapnia values only, $P < 0.05$).

nate all sensation, and since the adverse effects of anesthesia are dose dependent, the required dose can potentially induce severe cardio respiratory depression. Alternatively, anesthetic combinations often are used. The benefit is that an anesthetic which can eliminate all sensation except pain, at a low dose, can be combined with a specific analgesic agent which, alone, cannot induce complete anesthesia. The result is that both agents can be used to complement each other at low doses so that the adverse effects of high doses are avoided. In the study reported here, a faint limb withdrawal reflex was used to assess anesthesia (and indirectly analgesia) depth.

Effects of anesthesia on air breathing. Our results indicated that $\dot{V}_E$ was depressed by 50 to 60% using Saffan, pentobarbitone, and pentobarbitone plus Innovar Vet, but it was only depressed by 17% using ketamine/xylazine anesthesia. Ventilatory depression was principally due to a decrease in respiratory frequency. The $\dot{V}O_2$ was also depressed by a magnitude similar to, or less than that of $\dot{V}_E$. It is unlikely that the metabolic state of the animal was compromised by anesthesia since the arterial lactate concentration was not altered by any of the anesthetics tested. MABP was depressed equally by all anesthetics. Brown and co-workers (5) reported that anesthesia in general is a vasodepressor, although the magnitude of depression is independent of the dose and type of anesthetic used. The HR value reported for guinea pigs anesthetized with ketamine/xylazine (198 bpm) was lower than that for all other anesthetic regimens tested (270 bpm). Although it is an effective muscle relaxant, one of the adverse effects of xylazine is that it depresses HR. However, ketamine stimulates the central sympathetic tone, which promotes vasoconstriction (33). Consequently, the MABP for ketamine/xylazine-anesthetized guinea pigs (54 mmHg) was similar to that for all other groups of anesthetized guinea pigs (53 to 59 mmHg).

Despite the fact that ketamine/xylazine was the least depressive anesthetic, with regard to $\dot{V}_E$, arterial PaO_2 was similar for all anesthetics used (77 to 79 mmHg). However, the PaO_2 and $PaCO_2$ of all anesthetized guinea pigs were lower and higher, respectively, than that of conscious guinea pigs (98 and 33 mmHg, respectively). Brown and co-workers (5) reasoned that arterial gas tensions

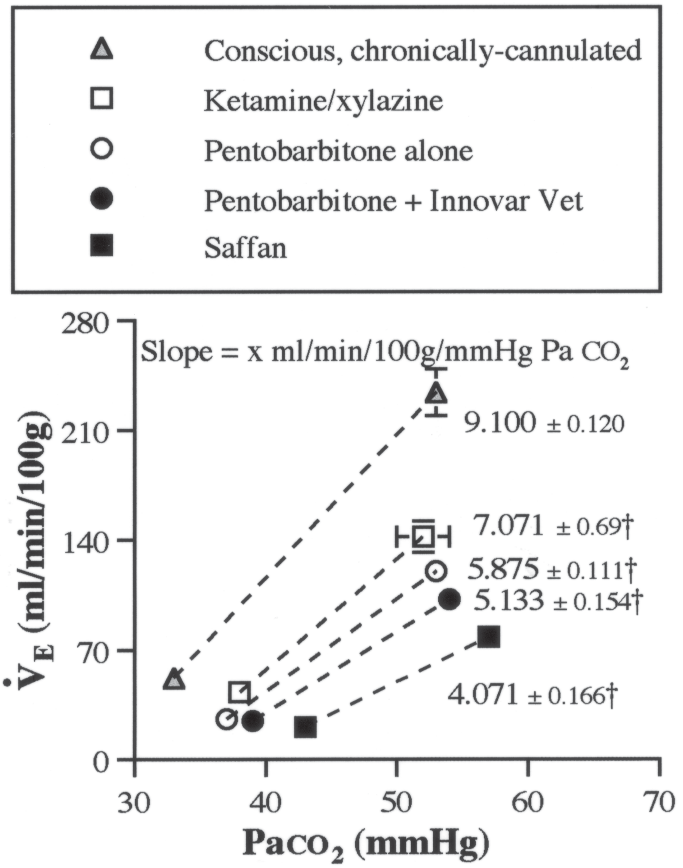

Figure 5. Ventilatory responsiveness (slope of the regression line for $\dot{V}_E$ versus $PaCO_2$) to hypercapnia (8% CO_2) of one conscious (cannulated) group (n = 11/group) and four anesthetized groups of guinea pigs (n = 7/group). The two data points provided represent air and hypercapnia. †Significantly different from the value for the conscious, long term-cannulated group (comparing the regression lines of each anesthetized group, $P < 0.05$).

would be adversely affected if the magnitude of respiratory depression was severe, and so reported that anesthesia, in general, would result in a reduction in PaO_2 and an increase in $PaCO_2$.

Saffan (alphadalone and alphaxalone) has not commonly been used for anesthetizing guinea pigs. We decided to test Saffan because it has been reported as a good anesthetic for the rat (23, 25). However, we found that the magnitude of $\dot{V}_E$ depression induced by Saffan (60%) was greater than that for any other anesthetic regimen we tested. Green and co-workers (18) and Brown and his colleagues (5) found that Saffan did not provide surgical anesthesia unless the dosage (40 to 45 mg/kg, i.m.) was higher than the manufacturer's recommended dosage of 36 mg/kg, i.m. Green and co-workers (18) also reported high mortality associated with high doses of Saffan. The dosage we used (infusion rate of 9.75 mg/kg/h) was necessary to induce sufficient anesthesia, and thus, lower doses could not be used. Consequently, Saffan induced the lowest breathing frequency, compared with data obtained for other anesthetic regimens.

Ketamine combined with xylazine has been frequently used for anesthetizing guinea pigs. Many investigators have reported ketamine/xylazine as the anesthetic of choice for procedures requiring light sedation (26) to those requiring surgical anesthesia (16, 19, 31). Ketamine has a wide therapeutic index (17), and is commonly combined with xylazine; the latter eliminates muscle tremors that result from use of ketamine alone (14, 17, 20, 26). Ketamine/xylazine anesthesia induced the smallest degree of $\dot{V}_E$ depression (17%) in our guinea pigs. The frequency reported by other investigators, including that in our guinea pigs (77 breaths/min), was partially determined by the dose administered. Barzago and co-workers (3) used a dose of 87 mg of ketamine plus 13 mg of xylazine/kg (i.e., 87/13 mg/kg), and reported a frequency of 44 breaths/min. This was similar to the frequency (49 breaths/min) reported by Radde and co-workers (26), who tested a dose identical to the dose we used. Radde and colleagues (26) and Brown and colleagues (5) tested lower dosages of ketamine/xylazine (35/5 mg/kg) and reported frequency values (64 breaths/min) that were comparable to those of the study reported here (77 breaths/min, with an infusion rate of 15/4 mg/kg/h).

Sodium pentobarbitone is a commonly used anesthetic. Most authors have used pentobarbitone dosages that are within a narrow therapeutic range (30 to 40 mg/kg, i.p.). Hoar (22) and Flecknell (14) suggested that the recommended dose of pentobarbitone for surgical anesthesia was close to the lethal dose and, therefore, the use of pentobarbitone should be avoided. The few studies in which pentobarbitone use has been reported indicated $\dot{V}_E$ depression. Blake and Banchero (4) reported a 43 to 50% depression in respiratory frequency for four pentobarbitone-anesthetized guinea pigs (35 mg/kg, i.p.). Consequently, $\dot{V}_E$ was depressed by 45% in two guinea pigs. In the other two guinea pigs, an increase in V_T counterbalanced the decrease in frequency so that $\dot{V}_E$ was not depressed. We have previously reported that pentobarbitone depresses $\dot{V}_E$ by 30% (28).

Pentobarbitone has weak analgesic properties and, thus, has been combined with phenoperidine/droperidol (12) or Innovar Vet (5); both drugs induce a state of neuroleptanalgesia (i.e., a state of tranquillity without anxiety or pain). The adverse effects of such drug combinations, however, are evident by the magnitude of respiratory depression, sometimes requiring mechanical ventilation (5, 12). When pentobarbitone was combined with Innovar Vet, the magnitude of ventilatory depression (52%) was similar to that when pentobarbitone was used alone (50%). The magnitude of frequency depression was also similar for both groups (52% decrease), and V_T was unaffected. Brown and co-workers (5) also tested the combination of pentobarbitone and Innovar Vet, and reported a frequency (53 breaths/min), which is greater than that reported by us (38 breaths/min), despite a larger dosage of Innovar Vet (0.4 mg/kg; *cf* 0.1 mg/kg in the study reported here). Body weight of their guinea pigs was lighter (230 g), and a respiratory frequency value was not reported for their conscious guinea pigs. Ventilatory data have not been reported where Innovar Vet has been used alone (27, 34).

Effects of anesthesia on the response to hypoxia. All anesthetic regimens attenuated, and altered the pattern of the $\dot{V}_E$ response to hypoxia. Increases in $\dot{V}_E$ were small and, unlike the conscious (V_T) response, the principal $\dot{V}_E$ component for anesthetized guinea pigs was respiratory frequency. Schwenke and Cragg (28) reported that the magnitude of the $\dot{V}_E$ response to hypoxia in pentobarbitone-anesthetized guinea pigs (30% increase) was similar to that reported for conscious guinea pigs (28% increase, unpublished data). However, the magnitude of the hypoxic $\dot{V}_E$ response in the five groups of conscious guinea pigs (one long-term cannulated and four uncannulated) was consistently a 45% increase. This highlights the depressive effects of anesthesia on the $\dot{V}_E$ response to hypoxia.

The magnitude of metabolic depression was generally not influenced by anesthesia, but it varied among the groups of guinea pigs (e.g., hypoxia depressed $\dot{V}O_2$ by 8% to 36% for the anesthetized groups). In anesthetized guinea pigs, hypoxia reduced MABP by 30% (Saffan) to 50% (ketamine/xylazine). Saffan has been the recommended anesthetic for the rat by some (23, 25), because, in animals under Saffan anesthesia, hypoxia "activates the brain-stem defence areas using peripheral chemoreceptor afferent input" (21, 32). Advantageously, the magnitude of hypoxia-induced hypotension is less severe. We found that the hypoxia-induced decrease in MABP was similar for conscious long term-cannulated (25%) and Saffan-anesthetized (30%) guinea pigs. However, the magnitude of hypotension induced by hypoxia also was statistically similar for guinea pigs anesthetized with Saffan and pentobarbitone, with or without Innovar Vet.

Effects of anesthesia on the response to hypercapnia. We found that the magnitude of the $\dot{V}_E$ response to CO_2, first, was similar between all anesthetic regimens (230 to 362%), and second, it appeared to be similar to the conscious response, probably because the $\dot{V}_E$ baseline had been reduced. However, all anesthetics tested significantly reduced the slope of the $\dot{V}_E$ versus $PaCO_2$ regression line. In this study, the effect of hypercapnia on $\dot{V}O_2$, MABP, and HR was negligible for conscious and anesthetized guinea pigs.

Conclusion. Our aim was to investigate four anesthetic regimens, with the best anesthetic being the one that caused the least magnitude of cardiorespiratory depression. Compared with those given to long term-cannulated guinea pigs, all anesthetics caused some degree of depression, whether it was cardiovascular, ventilatory, or both. However, we found that the results obtained from ketamine/xylazine-anesthetized guinea pigs were noticeably better than those obtained for all other regimens. Ketamine/xylazine depressed air breathing by only 17% (compared with 50 to 60% for all other regimens), and the ventilatory responsiveness to hypoxia and hypercapnia was least attenuated (using the conscious guinea pig as the control).

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