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THE EFFECTS OF CHLORALOSE-URETHANE AND SODIUM PENTOBARBITONE ANAESTHESIA ON THE LOCAL AND AUTONOMIC COMPONENTS OF THE CIRCULATORY RESPONSE TO ARTERIAL HYPOXIA

By P. I. KORNER,* G. LANGSFORD, D. STARR, J. B. UTHER,† W. WARD and S. W. WHITE‡

From the School of Physiology, University of New South Wales, Sydney, Australia

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SUMMARY

1. The circulatory and respiratory responses to severe arterial hypoxia were studied in normal rabbits, 'de-efferented' rabbits without functioning autonomic effectors, and atropinized animals before anaesthesia and during chloralose-urethane and sodium pentobarbitone anaesthesia. Net systemic autonomic activity and autonomic activity to the heart was assessed from a comparison of the responses of the various preparations.

2. In the normal spontaneously breathing animal each anaesthetic had a similar mode of action, and modified qualitatively the circulatory response present before anaesthesia. In the 'de-efferented' animal the circulatory response was determined by the local effects of hypoxia, and was altered only quantitatively during anaesthesia.

3. In the normal unanaesthetized animal the reflex changes in autonomic activity during hypoxia consisted of a large increase in vagal efferent activity, a decrease in cardiac sympatho-adrenal activity, and an increase in total sympatho-adrenal constrictor activity.

4 In hypoxia during anaesthesia the vagal efferent activity no longer increased, but the change in sympatho-adrenal activity to heart and systemic circulation was the same as before anaesthesia in the spontaneously breathing animal. During anaesthesia with controlled ventilation systemic sympatho-adrenal activity increased further, and brady-

* Present address: Cardiology Section, Department of Medicine, University of Sydney Sydney, Australia 2006.

† Research Fellow of the Life Insurance Medical Research Fund of Australia and New Zealand.

‡ Research Fellow of the National Heart Foundation of Australia.

cardia again developed. The bradycardia was now due exclusively to reduction in cardiac sympathetic activity and not to an increase in vagal efferent activity.

INTRODUCTION

Many studies in the intact animal have shown that anaesthetics modify the cardiovascular responses to physiological stimuli (Neil, Redwood & Schweitzer, 1949; Koppermann, Brendel & Thauer, 1955; Brown & Hilton, 1956; Heymans & Neil, 1958; Heymans, 1964; Gorten, Smith & Rushmer, 1964; Van Citters, Franklin & Rushmer, 1964) but the changes during anaesthesia in reflex autonomic effects or in the local effects which follow an induced physiological stimulus have not been analysed in detail. In the present experiments we have examined the changes during anaesthesia on the autonomic and local effector components of the response to arterial hypoxia. This response has been studied previously in the *unanaesthetized* rabbit and it has been found that the arterial chemoreceptors are the primary source of reflex activity and that secondary effects from the arterial baroreceptors and vagal afferents are also involved; on the efferent side there is a marked initial increase in vagal efferent activity, some reduction in cardiac sympathetic nerve activity, a sustained large increase in sympathetic constrictor nerve activity and a small rise in adrenal medullary hormone secretion, superimposed on the local vasodilator effects on the peripheral circulation (Korner & Edwards, 1960; Korner, 1965*a*; Chalmers, Korner & White, 1966; 1967*a*, *b*; Korner, Chalmers & White, 1967; Crocker, Johnson, Korner, Uther & White, 1968).

Chloralose-urethane and sodium pentobarbitone have been used in these experiments, because they are widely used in circulatory studies and are considered to have different effects on some cardiovascular reflex arcs (Price, 1960; Heymans, 1964; Greisheimer, 1965). The response to severe arterial hypoxia has been studied before and during anaesthesia in normal animals with intact reflexes, in atropinized animals without vagal efferent activity, and in 'de-efferented' animals without any autonomic effectors in which the local effects have been assessed.

METHODS

New Zealand White rabbits cross-bred with the New Zealand Giant strain were used in these experiments (mean body weight 2·6 kg; range 2·0–3·1 kg). The groups studied were normal animals, atropinized rabbits, and 'de-efferented' animals without functioning autonomic effectors. Details of the preparations, and of the methods used for determining cardiac output, arterial and right atrial pressures, heart rate, total peripheral resistance, respiratory minute volume and rate, and arterial P_{O_2}, P_{CO_2} and pH, have been described previously (Korner, 1965*b*; Chalmers, Isbister, Korner & Mok, 1965; White, 1966).

Following completion under local anaesthesia of the minor operative procedures of ear artery and right atrial catheterization and insertion of a tracheotomy tube, each unanaesthetized rabbit was placed into a rabbit box where it rested for 1 hr before commencing the first test. Each test was of 22 min duration and consisted of a control period breathing room air (7 min), a treatment period breathing a low O_2–N_2 mixture (10 min), and a recovery period breathing room air (5 min). One arterial sample was taken during the control period, and another towards the end of the period of hypoxia. Thirteen sets of observations of respiratory minute volume and rate, arterial and right atrial pressures, cardiac output and heart rate were obtained during the eight selected time intervals, as shown in Fig. 1. The mean value of each variable was obtained at these selected time intervals from the results of all animals in the group, and the standard error of the mean of the observations at a single time interval was calculated by analysis of variance as described previously (Chalmers *et al.* 1965). The average effects of treatment were expressed as percentages of the mean initial control value; absolute values of the latter are stated in the legend of each text figure. When the animals were anaesthetized with chloralose–urethane or sodium pentobarbitone, an approximately constant level of moderate surgical anaesthesia was maintained by injection of supplementary doses of anaesthetics as described in an accompanying paper (Korner, Uther & White, 1968). During those periods of each experiment in which the anaesthetized animals were ventilated artificially after administration of the muscle relaxant decamethonium iodide, care was taken to continue administration of chloralose or sodium pentobarbitone at the rate given to the animal while breathing spontaneously to ensure adequate maintenance of anaesthesia.

Three series of experiments were performed. In the first series the effects of severe arterial hypoxia were first determined in each rabbit before anaesthesia. Thirty minutes after completion of this test, the animal was anaesthetized with either chloralose-urethane or sodium pentobarbitone, and was placed in the prone posture of an unanaesthetized rabbit as described in an accompanying paper (Korner *et al.* 1968). The animals were warmed as required, using a table heater only between tests so that the reduction in body temperature observed in unanaesthetized animals (Korner, 1965*a*) also occurred during anaesthesia. The first test under anaesthesia commenced 60 min after induction, when the circulation had become stable (Korner *et al.* 1968), and the effects of hypoxia were determined during spontaneous respiration. Thirty minutes after the test, with anaesthesia being maintained at the same level, the animals were given decamethonium iodide (Korner *et al.* 1968) to produce muscular paralysis, and were ventilated with intermittent positive pressure using a Starling 'Ideal' pump. The respiration rate selected was 60/min and the minute volume 0·9–1·0 l./min, similar to mean resting values in the normal unanaesthetized animal. A third test was carried out, examining the effects of hypoxia during controlled ventilation.

In the second series of experiments two groups of animals were studied. In one group of unanaesthetized animals the effects of severe arterial hypoxia were determined as in the previous series. The animal was then fully atropinized (Korner *et al.* 1968) and the test repeated after 1 hr. The second group of animals was first anaesthetized with chloralose-urethane, and then studied in the same way before and after atropinization, whilst breathing spontaneously.

The third series of experiments was carried out during chloralose-urethane anaesthesia + decamethonium, entirely under conditions of controlled artificial ventilation while giving supplements of 2 ml. chloralose solution I.V. every 30–50 min (Korner *et al.* 1968). The response to hypoxia was examined before and after the administration of either atropine or propranolol, and after administration of atropine + propranolol (Chalmers *et al.* 1965).

RESULTS

Responses in normal and 'de-efferented' animals before and during anaesthesia

In these experiments the inspired gas mixtures were adjusted to produce a similar reduction in each animal in aterial P_{O_2}, before and during anaesthesia (Table 1). Anaesthesia modified the response to severe arterial

TABLE 1. Mean values ± standard error of the mean of arterial P_{O_2}, P_{CO_2}, and pH during the control period breathing air (C), and during the treatment period (T) 7 min after induction of hypoxia. Each animal was studied before anaesthesia (U), during anaesthesia breathing spontaneously (AS) and during anaesthesia with controlled ventilation (AC). Six normal animals and three 'de-efferented' animals were studied before and during chloralose-urethane anaesthesia; four normal rabbits and three 'de-efferented' animals were studied before and during sodium pentobarbitone anaesthesia

	U		AS		AC	
	C	T	C	T	C	T
Before and during chloralose-urethane anaesthesia						
Normal rabbits						
P_{O_2}	95 ± 2·4	32 ± 1·0	92 ± 1·1	32 ± 1·1	102 ± 3·3	33 ± 1·1
P_{CO_2}	36 ± 1·8	20 ± 1·2	32 ± 1·8	24 ± 2·1	32 ± 2·2	30 ± 1·4
pH	7·35 ± 0·017	7·47 ± 0·029	7·37 ± 0·019	7·46 ± 0·036	7·39 ± 0·024	7·41 ± 0·03
'De-efferented' rabbits						
P_{O_2}	104 ± 1·0	33 ± 1·0	91 ± 3·6	31 ± 1·5	90 ± 2·3	34 ± 1·2
P_{CO_2}	37 ± 1·8	22 ± 1·4	35 ± 2·1	29 ± 1·3	37 ± 1·0	38 ± 0·6
pH	7·40 ± 0·015	7·68 ± 0·01	7·51 ± 0·014	7·52 ± 0·01	7·45 ± 0·012	7·44 ± 0·017
Before and during sodium pentobarbitone anaesthesia						
Normal rabbits						
P_{O_2}	89 ± 1·7	33 ± 0·07	88 ± 0·8	33 ± 1·4	97 ± 5·1	33 ± 0·8
P_{CO_2}	38 ± 0·3	25 ± 1·7	37 ± 1·1	29 ± 1·4	34 ± 1·4	32 ± 0·6
pH	7·34 ± 0·019	7·49 ± 0·034	7·37 ± 0·02	7·47 ± 0·04	7·37 ± 0·033	7·42 ± 0·027
'De-efferented' rabbits						
P_{O_2}	101 ± 2·5	34 ± 0·5	88 ± 4·0	31 ± 0·5	89 ± 1·0	34 ± 1·5
P_{CO_2}	30 ± 1·0	21 ± 1·0	34 ± 2·0	25 ± 0·8	28 ± 3·0	27 ± 3·2
pH	7·47 ± 0·02	7·52 ± 0·03	7·39 ± 0·01	7·67 ± 0·06	7·39 ± 0·03	7·46 ± 0·04

hypoxia in both normal and 'de-efferented' animals, and the effects were almost identical with each anaesthetic. During anaesthesia in normal animals breathing spontaneously, the magnitude of the respiratory response to hypoxia became attenuated, and the circulatory response was altered qualitatively. In contrast with the findings in the unanaesthetized animal, where there was bradycardia and transient reduction in cardiac output, the heart rate did not change significantly during anaesthesia and the cardiac output rose (Figs. 1 and 2). Some bradycardia and reduction in cardiac output were observed again in each animal during controlled artificial ventilation. In the 'de-efferented' animals without functioning autonomic effectors, the magnitude of the respiratory response was also diminished during anaesthesia but the circulatory effects remained qualitatively

similar (Figs. 3 and 4). The role of the autonomic nervous system in the responses before and during anaesthesia has been assessed by contrasting the responses of normal and 'de-efferented' animals.

Respiration. In normal unanaesthetized rabbits the respiratory minute volume increased during hypoxia to an average of 220 % of control, and the rate to 180 % of control (Figs. 1 and 2). The spontaneous resting respiration was somewhat depressed by anaesthesia (Korner *et al.* 1968), as was the response to arterial hypoxia: the respiratory minute volume

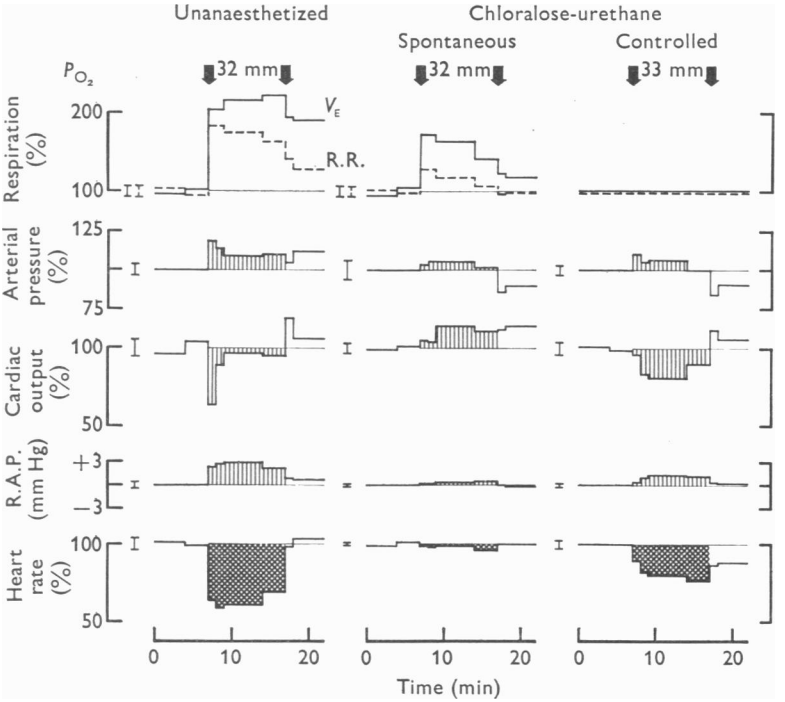

Fig. 1. Mean effects on respiration and circulation of arterial hypoxia in six normal rabbits before and during chloralose-urethane anaesthesia. Each animal was studied before anaesthesia (left panel), during anaesthesia whilst breathing spontaneously (middle panel), and during anaesthesia with controlled respiration (right panel). Low O_2 mixtures inhaled between arrows, room air at other times and arterial P_{O_2} values during hypoxia are shown. Respiration (minute volume ($\dot{V}_E$)—continuous line; rate (R.R.)—dashed line), arterial pressure, cardiac output and heart rate expressed as % of initial control, right atrial pressure (R.A.P.) as change from control in mm Hg. Symbol to the left of each variable is twice the standard error of the mean of a single time interval. The absolute mean initial control values are given below in the order unanaesthetized, anaesthetized-spontaneous respiration, anaesthetized-control respiration: (*a*) respiration-ventilation = 1·03, 0·81, 0·95 l./min; rate = 62, 40, 60/min, (*b*) arterial pressure = 98, 93, 94 mm Hg, (*c*) cardiac output = 541, 598, 546 ml./min, (*d*) R. atrial P = −0·2, +0·2, +2·4 mm Hg, (*e*) heart rate = 269, 321, 331/min.

rose to only 165 % of control, and respiration rate to only 120 % of control. The degree of hypocapnia during hypoxia was less marked than before anaesthesia owing to the diminution in respiratory response (Table 1). In the 'de-efferented' rabbits, there was approximately the same degree of reduction in the respiratory response during anaesthesia as in normal animals (Figs. 3 and 4).

Heart rate. In the unanaesthetized normal animals the heart rate fell on the average to 65 % of control during the period of hypoxia. During anaesthesia in the spontaneously breathing animal, although the blood

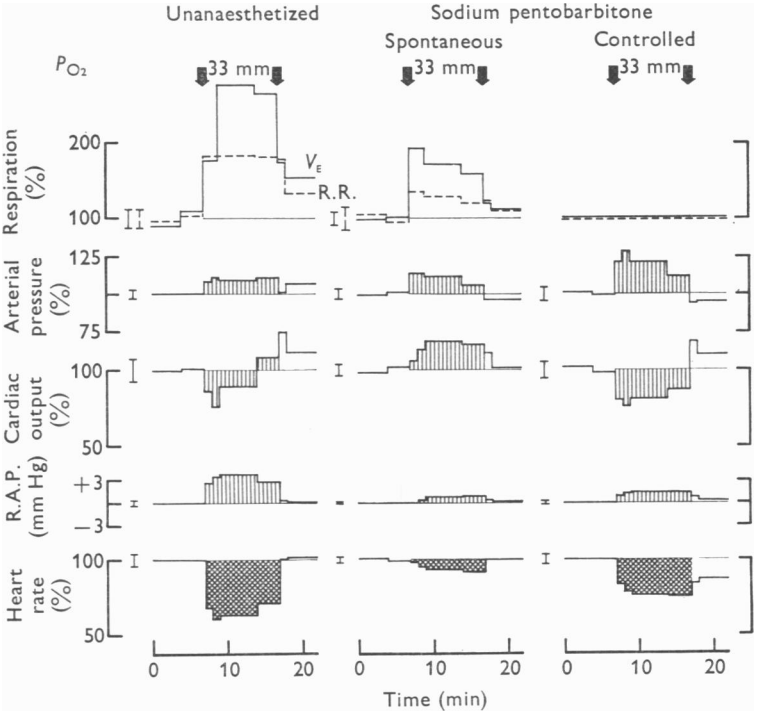

Fig. 2. Mean effects on respiration and circulation of arterial hypoxia in four normal rabbits before and during sodium pentobarbitone anaesthesia. Procedure and notation as in Fig. 1. The absolute mean initial control values during the three control periods are from left to right panel, (*a*) respiration-ventilation = 0·84, 0·61, 0·95 l./min; rate = 56, 38, 60/min, (*b*) arterial pressure = 89, 89, 98 mm Hg, (*c*) cardiac output = 576, 592, 518 ml./min, (*d*) R. atrial P = 0, +0·5, +2·7 mm Hg, (*e*) heart rate = 275, 302, 296 beats/min.

pressure response was unchanged, the bradycardia was completely abolished with chloralose-urethane anaesthesia, and was greatly reduced with sodium pentobarbitone where the heart rate fell only by 5 % (P = 0·05) (Figs. 1 and 2). During anaesthesia with controlled ventilation

some fall in heart rate (to 80% of control; $P < 0{\cdot}001$) again occurred during hypoxia. The net reflex chronotropic response observed in the unanaesthetized animal is thus virtually abolished by both anaesthetics, but reappears when ventilation is controlled. The abolition or reduction of the bradycardia was not the result of block of vagal endings by the anaesthetic (with or without decamethonium), since electrical stimulation of the cardiac end of the cut vagus could readily produce graded degrees

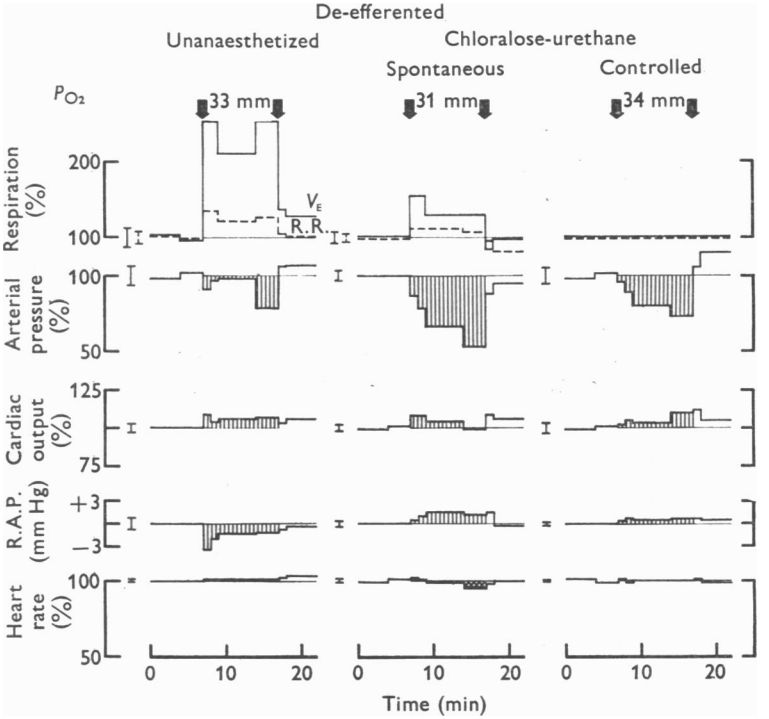

Fig. 3. Mean effects on respiration and circulation of arterial hypoxia in three 'de-efferented' rabbits before and during chloralose-urethane anaesthesia. Procedure and notation as in Fig. 1. The absolute mean initial control values during the three control periods are from left to right panel, (*a*) respiration-ventilation = 1·0, 0·80, 0·95 l./min, rate = 84, 50, 60/min, (*b*) arterial pressure = 74, 68, 63 mm Hg; (*c*) cardiac output = 506, 655, 573 ml./min, (*d*) R. atrial P = +0·6, +0·5, +1·9 mm Hg, (*e*) heart rate = 221, 230, 231 beats/min.

of cardiac slowing (Fig. 5). Stimulation of the nerve was not performed in the unanaesthetized animal, so that some alteration in peripheral sensitivity due to anaesthesia could not be excluded (cf. Gruber & Keyser, 1946).

The heart rate of animals without functioning autonomic effectors did not change significantly during hypoxia either before or during anaesthesia

(Figs. 3 and 4). The local chronotropic effects of hypoxia are thus small, and virtually unaffected by anaesthesia during the maintenance phase.

Haemodynamic findings. During hypoxia in the normal unanaesthetized animals, there was a rise in arterial pressure, reduction in cardiac output and significant elevation in right atrial pressure associated with the bradycardia (Figs. 1 and 2). During anaesthesia + spontaneous ventilation the arterial pressure again increased during hypoxia but there was now a significant increase in cardiac output with both anaesthetics to 113 % of

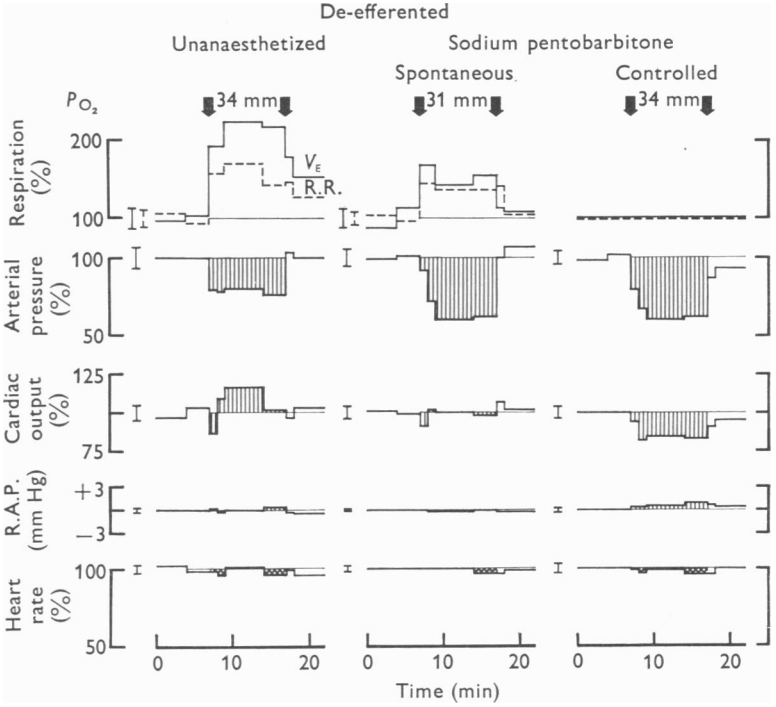

Fig. 4. Mean effects of arterial hypoxia in three 'de-efferented' rabbits before and during sodium pentobarbitone anaesthesia. Protocol and notation as in Fig. 1. The absolute mean initial control values during the three control periods are from left to right panel, (*a*) Respiration-ventilation = 0·97, 0·78, 0·95 l./min, rate = 78, 64, 60/min, (*b*) arterial pressure = 72, 65, 57 mm Hg, (*c*) cardiac output = 484, 642, 500 ml./min, (*d*) R. atrial P = +0·8, +1·1, +2·7 mm Hg, (*e*) heart rate = 224, 220, 218 beats/min.

control, and little change in right atrial pressure (with abolition of bradycardia). During anaesthesia + controlled ventilation hypoxia resulted in a rise in arterial pressure, reduction in cardiac output and again a rise in right atrial pressure.

In the unanaesthetized 'de-efferented' rabbit the arterial pressure fell to 85 % of control during the period of hypoxia, and there was a small rise

in cardiac output (Figs. 3 and 4). During anaesthesia and spontaneous respiration, the fall in arterial pressure during hypoxia was greater, falling to an average of 70 % of control with both anaesthetics, and there was little change in cardiac output. During anaesthesia and controlled ventilation the response to hypoxia differed with the two anaesthetics, with a greater fall in arterial pressure and significant reduction in cardiac output observed with sodium pentobarbitone (cf. Figs. 3 and 4, third panels). The changes in total peripheral resistance during hypoxia have been plotted in Fig. 6 for both normal and 'de-efferented' animals. In the unanaesthetized normal animal the total peripheral resistance rose strikingly during

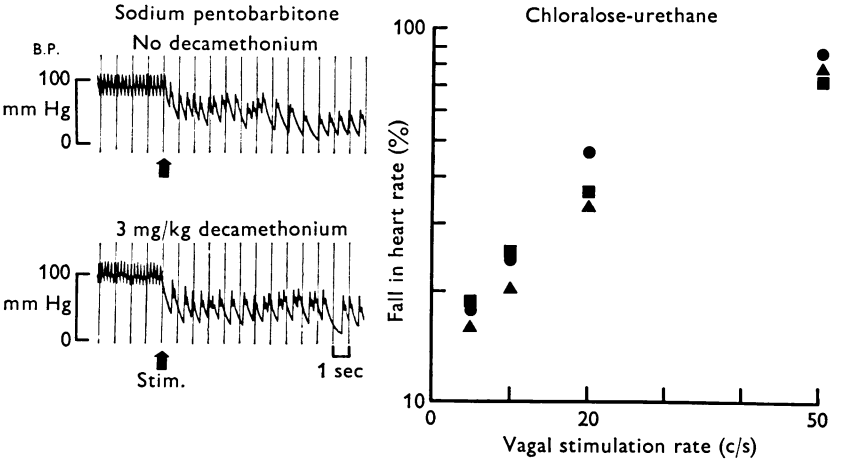

Fig. 5. (Left panel), records of changes in carotid artery pressure, in a rabbit anaesthetized with sodium pentobarbitone, obtained by electrical stimulation of the peripheral end of the cut cervical vagus (at arrow) with rectangular pulses of 10 V amplitude, 1 msec duration at 50 c/s. The upper record was obtained before, and the lower record after, administration of 3 mg/kg decamethonium iodide. (Right panel), results of graded stimulation of the peripheral end of cut cervical vagus in another animal anaesthetized with chloralose-urethane, using rectangular pulses of 10 V amplitude, 1 msec duration and varying frequency. The drop in heart rate after 10 sec stimulation was expressed as % of the preceding control value ($333 \pm 3{\cdot}6$ (S.E. of mean) beats/min). ●, no decamethonium, ■, 1 mg/kg decamethonium, ▲, 3 mg/kg decamethonium.

the early phase of hypoxia to about 170 % of control, and after a brief period stabilized at a level slightly above control values for the rest of the treatment period. In the spontaneously breathing anaesthetized animals the early rise in total peripheral resistance was absent, and the resistance fell to a value slightly below initial control. In the 'de-efferented' animals the total peripheral resistance fell during hypoxia, suggesting a local vasodilator response (Chalmers *et al.* 1967*b*), and the fall was greatest

during anaesthesia in the spontaneously breathing animal, differing significantly ($P = 0{\cdot}05$) from the fall before anaesthesia. However, the *difference* in the change of total peripheral resistance between the normal and 'de-efferented' animal at the end of hypoxia, was almost the same as observed in the same animal before anaesthesia. During anaesthesia + controlled ventilation, the mean rise in total peripheral resistance was greater than before anaesthesia, and the difference between corresponding normal and 'de-efferented' changes was maximal.

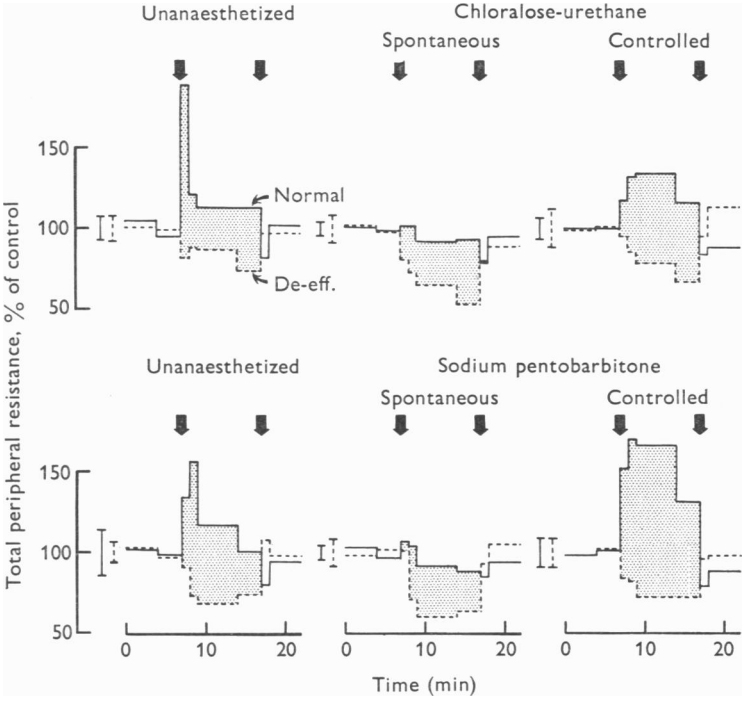

Fig. 6. Mean effects of arterial hypoxia (between arrows) on the total peripheral resistance before and during anaesthesia in normal animals (continuous lines) and 'de-efferented' animals (interrupted lines). Total peripheral resistance is expressed as a percentage of the mean initial control value in each group. Effects of chloralose-urethane shown in top row, and effects of sodium pentobarbitone in lower row. Notation otherwise as in Fig. 1. The shaded area during the treatment period delimited by the responses of normal and 'de-efferented' animals is a function of reflex autonomic activity as discussed in the text.

The effects of atropine

In the unanaesthetized normal animal blocking vagal efferents by means of atropine diminished the bradycardia during hypoxia, and altered the haemodynamic response (Fig. 7, left two panels). On the other hand, in the

anaesthetized rabbit breathing spontaneously, some bradycardia appeared during hypoxia only after atropinization, but there was little change in the haemodynamic findings (Fig. 7, right two panels). The respiratory

TABLE 2. Mean respiratory and arterial blood findings before (C) and during arterial hypoxia (T) in six normal unanaesthetized rabbits before and after atropinization; also in five normal rabbits anaesthetized with chloralose-urethane before and after atropine. The result from each rabbit is the mean of all observations during the whole control and treatment period. Standard error of the difference calculated from within animal comparisons

	Before atropine			After atropine		
Unanaesthetized	C	T	S.E. diff.	C	T	S.E. diff.
Respiration rate (breaths/min)	64	100	±5·0	84	107	±4·9
Respiration minute volume (l./min)	0·88	1·88	±0·10	1·10	2·05	±0·10
P_{O_2}	97	32	±2·3	101	30	±2·6
P_{CO_2}	37	24	±2·0	34	23	±2·1
Chloralose-urethane						
Respiration rate (breaths/min)	35	41	±1·8	41	45	±2·1
Respiration minute volume (l./min)	0·46	0·78	±0·08	0·57	0·78	±0·05
P_{O_2}	87	32	±2·9	95	31	±3·8
P_{CO_2}	43	30	±2·4	39	32	±2·8

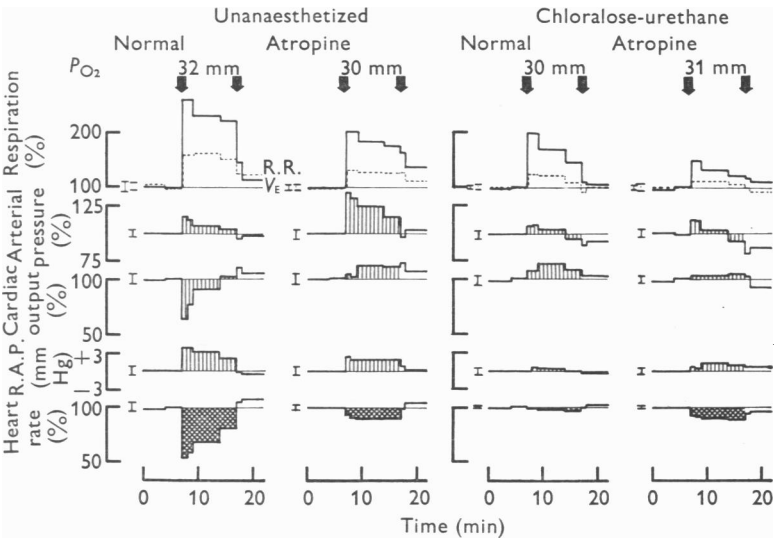

Fig. 7. Mean effects on respiration and circulation of hypoxia before and after atropinization in six normal unanaesthetized rabbits (left two panels), and in five normal animals anaesthetized with chloralose-urethane (right two panels). Procedure and notation as in Fig. 1. Absolute initial control values are listed for each variable in the order of panels 1–4, (*a*) respiration-ventilation = 0·88, 1·10::0·46, 0·57 l./min, rate = 64, 84::35, 41/min, (*b*) arterial pressure = 110, 102::97, 88 mm Hg, (*c*) cardiac output = 596, 585::526, 480 ml./min, (*d*) R. atrial P = 0·9, −1·6::−1·5, −1·6 mm Hg, (*e*) heart rate = 277, 309::298, 316 beats/min.

response expressed as percentage of control was diminished after atropine (Fig. 7), but the absolute values of the respiratory variables were the same during hypoxia as before atropine (Table 2). Since atropine stimulates the resting respiration in both unanaesthetized and anaesthetized animals, the apparent diminution in respiratory response is probably not a physiologically significant effect.

Heart rate. In the unanaesthetized animal the heart rate fell during hypoxia by only about 10% (i.e. 30 beats/min) after giving atropine, compared with an average fall of 35% (i.e. 100 beats/min) observed in the same animal before atropine. The bradycardia of the unanaesthetized

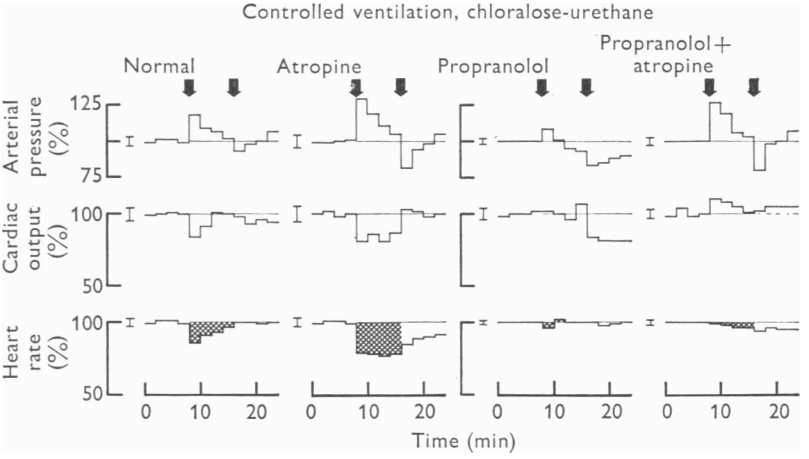

Fig. 8. Mean circulatory effects of arterial hypoxia (between arrows) in animals ventilated during chloralose-urethane anaesthesia with the ventilation controlled at the resting value before and after giving atropine (three rabbits), and in another group after giving propanolol, and propranolol + atropine (three rabbits). Notation as in Fig. 1. Absolute initial control values are given in the order panels 1–4, (*a*) arterial pressure = 108, 99::109, 90 mm Hg, (*b*) cardiac output = 520, 456::530, 430 ml./min, (*c*) heart rate = 300, 307::200, 220 beats/min.

animal during hypoxia is therefore produced by the summed effects of increased vagal efferent activity and reduced sympathetic activity to the heart. During chloralose-urethane anaesthesia with spontaneous ventilation there was no significant change in heart rate during hypoxia before atropine, but after atropine the heart rate fell during the treatment period by about 8% ($P < 0{\cdot}01$). The minimal changes in heart rate under anaesthesia + spontaneous ventilation therefore result from the opposing influences of reduction in vagal efferent activity and reduction in cardiac sympathetic activity.

During controlled ventilation there was again no increase in vagal efferent activity during hypoxia but only reduction in cardiac sympathetic

activity, since the bradycardia was even more marked after atropine than in the spontaneously breathing animal, but was abolished by propranolol (Fig. 8). In view of the findings of Scott (1966) in rabbits anaesthetized with urethane only, of an increased vagal efferent activity during perfusion of the isolated carotid chemoreceptors with hypoxic blood (see Discussion), two additional experiments were carried out in which the effects of arterial hypoxia on the heart rate were examined during controlled ventilation, with animals anaesthetized with urethane only (dose, 1 g/kg). In these experiments the heart rate fell by the same amount as in the chloralose-urethane experiments during controlled ventilation, and the bradycardia became more marked after administration of atropine (1 expt.), and after complete bilateral mid-cervical vagotomy (1 expt.), and appears thus to have resulted entirely from reduction in cardiac sympathetic activity.

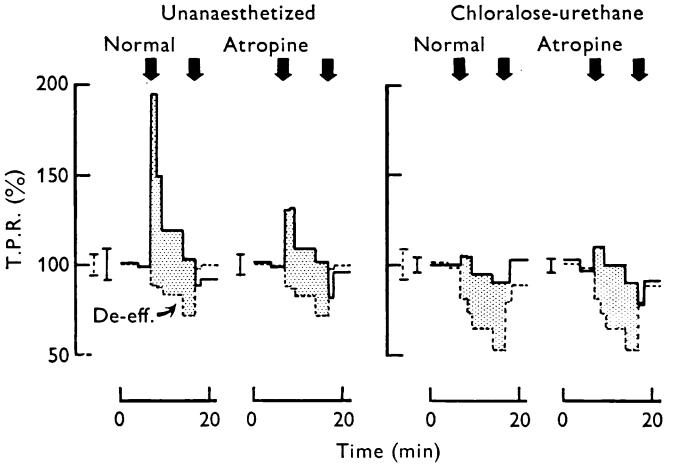

Fig. 9. Mean effects of arterial hypoxia (between arrows) on the total peripheral resistance, expressed as percentage of the mean initial control value, before and after atropinization (continuous lines) in six unanaesthetized and five anaesthetized animals (data from Fig. 7). The results have been compared with findings in 'de-efferented' animals (interrupted lines) studied under identical conditions. The area between the two lines during the treatment period is a function of autonomic activity as discussed in the text.

Haemodynamic findings. In the unanaesthetized animal, where the degree of bradycardia during hypoxia was much less after atropine, the transient reduction in cardiac output before atropine was converted to an immediate rise, the increase in right atrial pressure was less marked and there was a greater rise in arterial pressure (Fig. 7, left two panels). Under chloralose-urethane anaesthesia, where atropinization was associated with reappearance of some bradycardia, there was a less marked rise in cardiac output and a greater rise in right atrial pressure (Fig. 7, right two panels).

The changes in total peripheral resistance are shown in Fig. 9, with the response of the appropriate 'de-efferented' groups providing the base line for the local effects of hypoxia. In the unanaesthetized normal animal the early transient rise in total peripheral resistance was greatly reduced by atropine, but during the latter part of treatment the total peripheral resistance was the same before and after atropine. Under chloralose-urethane anaesthesia the changes in total peripheral resistance values during hypoxia were identical before and after atropine.

DISCUSSION

Assessment of autonomic activity. In the normal animal, with intact reflexes, the circulatory response to arterial hypoxia is determined by the interaction of mechanical and local tissue factors with the autonomic reflex effects. In the 'de-efferented' animal the neural control loop has been broken and the circulatory response is determined by 'local' mechanisms alone, assuming that no other rapidly acting compensating systems develop.

From a comparison of the responses of these two preparations a 'transfer' function describing the activity of the autonomic nervous system in terms of the input disturbance (hypoxia) can be derived. In the present analysis we make the simplifying assumption that at a given level of arterial P_{O_2}, P_{CO_2} and pH the mechanical and local tissue effects during hypoxia act in a similar way in both normal and 'de-efferented' animals. Carrier, Walker & Guyton (1964), and Guyton, Ross, Carrier & Walker (1964) have shown that the conductance changes during hypoxia are closely similar in small vessels isolated from the surrounding tissue, and in organs perfused with hypoxic blood. They have suggested that the dilator effect of hypoxia results from the direct effects on the vascular smooth muscle cells rather than from indirect actions due to the production of tissue metabolite intermediates. The studies of Uchida, Bohr & Hoobler (1967) have demonstrated that characteristic sensitivities of different vascular beds to other vasoactive agents are a property of the isolated small vessels themselves. This model of *direct* local vascular action will make the local effects of brief periods of hypoxia *relatively* independent of the degree of neural vasomotor tone, and provides a reasonable basis for assuming similarity of local effects in both preparations.

The determination of the 'transfer' function will be simplified further if circulatory base line conditions in both preparations are similar, if 'de-efferentation' does not produce non-specific effects on vessel sensitivity, and if both preparations remain stable during treatment. All these conditions are satisfied approximately in the present experiments. Thus

the differences in vascular tone between the preparations are small (Chalmers *et al.* 1967*b*, *c*), the total peripheral resistance of the 'de-efferented' animals being about 80–90% of normal. This difference in resting conditions can probably be neglected in assessing the magnitude of the normal vasodilator response from the findings in 'de-efferented' animals. This is suggested from the comparison of the responses during hypoxia of 'de-efferented' animals with animals with section of the carotid sinus and aortic nerves in which the resting vascular tone is considerably higher and the neural control loop has been broken at the input end (Chalmers *et al.* 1967*b*). The time course of the circulatory changes is identical in both areflexic preparations, and despite the considerable differences in resting conditions the magnitude of the fall in regional vascular resistance is only *slightly* greater in animals with section of the afferent nerves. The autonomic effectors remain structurally intact in the latter group so that the results also indicate that the process of 'de-efferentation' does not lead to non-specific changes in circulatory sensitivity to hypoxia. It is unlikely that other rapidly acting control systems play a part in the responses of the 'de-efferented' animal, since the magnitude of the vasodilator response in various regions is approximately the same as in preparations with more restricted local autonomic ablations (Chalmers *et al.* 1966; Korner *et al.* 1967) or in isolated beds during perfusion with hypoxic blood. The circulation of the 'de-efferented' animal remains reasonably stable for about 20–30 min (Korner *et al.* 1967). With adequate stability in both normal and 'de-efferented' preparations the magnitude of autonomic activity becomes a relatively simple function related to the difference in the mean responses of the two preparations. The mean response of the 'de-efferented' animal serves as a base line for estimating the local effects, whilst the normal animal shows the superimposed local and autonomic effects.

In the same way the difference in the responses of normal animals and those with selective block of a single autonomic effector pathway will permit assessment of the normal role of the blocked component in the integrated response. The question of alternative compensation is very relevant, since the selective block must not produce haemodynamic changes which would greatly alter the activity of the remaining autonomic effectors as a result of altering the input from the various cardiovascular receptors.

In the present experiments the heart rate changes have been used to analyse changes in cardiac autonomic activity during hypoxia. Total peripheral resistance changes during hypoxia depend on cardiac as well as peripheral circulatory factors, since the ratio includes information about cardiac output and mean pressure gradients. Total peripheral resistance

is thus a convenient variable for assessing changes in total peripheral systemic autonomic activity.

Cardiac autonomic activity. The responses of the 'de-efferented' animal demonstrate that the local chronotropic effects on the heart of hypoxia and anaesthesia are minimal, so that in the normal animal the heart rate changes are all the result of reflex activity. Absence of local cardiac chronotropic effects does not rule out some loss of myocardial contractile force, and this becomes apparent during hypoxia under sodium pentobarbitone anaesthesia and controlled ventilation (Fig. 4).

The results in the normal unanaesthetized rabbit confirm previous observations that there is a marked increase in efferent vagal activity and a smaller reduction in cardiac sympathetic activity, both contributing to the fall in heart rate (Korner & Edwards, 1960). During anaesthesia the increase in vagal activity is completely eliminated, but the magnitude of the cardiac sympathetic withdrawal response is the same as before anaesthesia. In fact, during anaesthesia there is a *decrease* in vagal activity tending to increase heart rate and cancelling the effects on heart rate of reflex reduction in cardiac sympathetic activity. During controlled ventilation vagal efferent activity does not increase and the greater bradycardia is entirely the result of unmasking of the reduced cardiac sympathetic activity. The predominant effects of chloralose-urethane and sodium pentobarbitone anaesthesia are probably not the result of a peripheral action of these drugs on the vagal endings (Fig. 5, Gruber & Keyser, 1946), but must be due to their action on the central nervous system and/or the arterial chemoreceptors.

The absence of an increase in vagal efferent activity during hypoxia in the intact, anaesthetized rabbit differs from the results obtained with the same anaesthetics during perfusion of the isolated arterial chemoreceptors with hypoxic blood in the dog, cat and rabbit whilst these animals were normally ventilated with air (Daly & Scott, 1958, 1963; Downing, Remensnyder & Mitchell, 1962; Daly & Hazzledine, 1963; MacLeod & Scott, 1964; Scott, 1966; Daly & Ungar, 1966). In all perfusion studies a reflex increase in vagal efferent activity has been demonstrated. The discrepancy in the findings could be the result of greater central nervous depression of reflex vagal efferent activity by anaesthesia + hypoxia in the present study, compared with depression by anaesthesia alone in the perfusion experiments in which the central nervous system remains normoxic. Alternatively, some ischaemia of the suprabulbar regions of the nervous system could result from ligation of the carotid arteries during isolation of the chemoreceptors, despite the anastomoses between the vertebral and carotid systems (Chungcharoen, Daly, Neil & Schweitzer, 1952). This could modify selectively the threshold for one or more components of the auto-

nomic reflex response by producing complex autonomic release and stimulation phenomena.

Systemic autonomic activity. In the 'de-efferented' animal the fall in total peripheral resistance during hypoxia is greater in the anaesthetized than the unanaesthetized animal. This could be due to potentiation of the local dilator response to hypoxia either by the higher P_{CO_2} levels present even during spontaneous respiration, or by the direct action of the anaesthetics. These factors have not been analysed further in the present study.

The marked initial rise in total peripheral resistance, observed in the unanaesthetized animal during the early part of hypoxia, is eliminated under anaesthesia in the spontaneously breathing animal. An almost identical diminution of the early rise in total peripheral resistance is observed in the unanaesthetized animal after atropinization. On the other hand, in the anaesthetized animal atropine has no effect on the changes in total peripheral resistance during hypoxia. It seems likely that the bradycardia, which is eliminated by both anaesthetics and atropine, contributes to the initial rise in total peripheral resistance in the normal, unanaesthetized animal. This bradycardia will immediately reduce cardiac output by diminishing emptying, whilst the right atrial pressure increases. This will result in a transient damming back of blood owing to the great distensibility of the venous system. When the venous capacity reaches equilibrium, the cardiac output will again increase towards normal. The results before and after atropine suggest that the early changes in total peripheral resistance in the unanaesthetized animal are largely the result of reflex secondary activity following the cardiac parasympathetic effects on cardiac output. It is unlikely that atropine exerts its action in the unanaesthetized rabbit in any other way than by blocking vagal efferents: for example, the absolute respiratory response to hypoxia is unaltered (Table 2), and in the doses used atropine does not affect the arterial chemoreceptor discharge rate (Landgren, Liljestrand & Zotterman 1952; 1953; Anichkov & Belen'kii, 1963; Eyzaguirre & Koyano, 1965); the effects on ganglion transmission in sympathetic ganglia is probably physiologically unimportant during maintenance of the normal nicotinic component of ganglionic transmission (Brown 1967). Furthermore, a cholinergic vasodilator innervation is apparently absent in the rabbit (Uvnäs, 1967).

During the later part of hypoxia the difference between the total peripheral resistance of the normal and 'de-efferented' animal is not affected by atropinization in either unanaesthetized or anaesthetized animals (Fig. 9), and thus represents the 'steady state' increase in net reflex systemic sympatho-adrenal activity during hypoxia. Previous peripheral blood flow studies suggest that this increase is largely the result of an

increase in peripheral sympathetic constrictor activity (Chalmers *et al.* 1966, 1967*b*). Anaesthesia thus does not alter cardiac sympatho-adrenal activity or the 'steady-state' level of systemic sympatho-adrenal activity. The virtual identity of sympatho-adrenal response before and during anaesthesia may be somewhat misleading, since the stimulus to the arterial chemoreceptors is greater during anaesthesia than before, due to the smaller degree of hypocapnia (Eyzaguirre & Lewin, 1961; Neil & Joels, 1963).

During controlled ventilation under anaesthesia the difference in total peripheral resistance values between normal and 'de-efferented' animals is again of sympatho-adrenal origin, and is greater than the sympatho-adrenal component of the responses before anaesthesia, or during anaesthesia with spontaneous respiration. The greater autonomic effect is probably accounted for by the greater chemoreceptor stimulus (Table 1), and by the absence of the normally acting inhibitory vagal afferent effects related to hyperventilation (Crocker *et al.* 1968).

The effects of anaesthesia on the respiratory and circulatory response to arterial hypoxia are thus very selective. Chloralose-urethane and sodium pentobarbitone affect selectively respiration rate, and eliminate reflex cardiac parasympathetic activity without greatly altering sympatho-adrenal activity to the heart and periphery. The similarity of the effects of these two anaesthetics on the reflex response suggests that they have a similar mode of action on the central nervous mechanisms controlling the circulation and respiration.

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