Dynamic Characteristics of the Cardiovascular Autonomic Effects during Severe Arterial Hypoxia in the Unanesthetized Rabbit

By Paul I. Korner, M.D., and John B. Uther, M.B.

ABSTRACT

The reflex autonomic effects due to inhalation of low concentrations of oxygen (Pao$_2$ 30 mm Hg) were assessed in unanesthetized rabbits from the differences in responses between groups of normal and autonomically "de-efferented" rabbits and those with selective effector block. Changes due to autonomic effects were estimated for heart rate, total and regional (portal, renal, muscle, and cutaneous) peripheral resistance, and the rate of epinephrine secretion. The autonomic effects could be described by an early component having a time course similar to the initial vagal effects, where the maximum change occurred soon after the start of hypoxia and then declined to 37% of this value in 5 minutes, and a late component similar to the neural effects in muscle, starting 5 minutes after the start of hypoxia and rising slowly to 63% of the maximum in 15 minutes. Four autonomic effector patterns involving different combinations of early and late components were distinguished: (1) vasoconstriction in the portal and renal beds followed the time course of the sum of early and late components; (2) inhibition of cutaneous constrictor tone was the mirror image of the above response; (3) the biphasic sympathoadrenal effect on heart rate was the sum of a negative early and positive late component; (4) the neural constrictor effect in muscle and the rate of epinephrine secretion had only a late component. The early effects resulted in reduction in cardiac output and major redistribution of peripheral blood flow, probably due to chemoreceptor stimulation. During the late phase, cardiac output rose and blood flow was redistributed further, probably mainly through baroreceptor mechanisms.

ADDITIONAL KEY WORDS

- arterial chemoreceptors
- baroreceptors
- peripheral autonomic effects
- blood flow distribution
- control system

The cardiovascular response to a generalized disturbance such as severe arterial hypoxia is due partly to the reflexly evoked autonomic effects and partly to the local effects of hypoxia on the heart and peripheral circulation. The local effects produce gradual changes in the metabolic state of the heart and peripheral circulation during prolonged hypoxia, while the reflex effects depend on the acuteness of the hypoxic stress, on possible adaptations in the receptors and areas of the central nervous system, and on changes in stimulus profile due to the local effects. The reflex autonomic effects can be determined directly in experiments under anesthesia, but anesthesia interferes with the function of the central nervous system (1, 2). In this paper we have examined in the unanesthetized rabbit the time course during severe hypoxia of the autonomic effects on the heart and peripheral circulation including portal, renal, muscle, and cutaneous vascular beds. The autonomic effects have been assessed from the...
difference in responses to an identical change in arterial blood composition between normal animals with intact reflexes, and autonomically "de-efferented" animals with a normal respiratory response to hypoxia (2, 3). We have also examined the contribution of individual effector components using selective autonomic block.

Methods

The experiments were performed on offspring of New Zealand White rabbits crossbred with the New Zealand Giant strain; average weight was 2.6 kg (range 2.0 to 3.5 kg).

Operations.—In animals with intact autonomic effectors, a thermistor catheter for measuring cardiac output was inserted into the upper abdominal aorta via the iliolumbar artery 3 to 7 days before an experiment (4). For measuring peripheral blood flow, in another group of animals, special polyvinyl chloride double-lumen local thermodilution catheters were inserted into the left renal, portal, and common iliac veins 4 to 10 days before an experiment (5, 6).

Autonomically "de-efferented" rabbits were prepared 10 to 14 days before an experiment by performing bilateral adrenalectomy at the same operation at which aortic or local thermodilution catheters were inserted (7). In the rabbit, steroid maintenance requires therapy with both cortisone acetate (1.0 mg/day im) and deoxycorticosterone acetate (1.5 mg/day im) (8, 9). The doses were determined in a metabolic study that maintained postoperative hematocrits, blood urea, serum Na and K close to their preoperative values, while the animals’ food and water consumption rapidly returned to normal (9). Treatment with guanethidine sulfate (12.5 mg/kg/day iv) began four days after adrenalectomy, this dose completely blocking sympathetic nerve transmission to the hindlimb (10); in normal rats and rabbits, this dosage depletes tissue catecholamine stores except in the central nervous system and adrenal medulla (11-13). The adrenalectomized guanethidine-treated animals were atropinized on the day of the experiment.

In another group of rabbits, bilateral adrenalectomy was performed 4 to 7 days before the experiment, and the left lumbar sympathetic chain removed from L3 to the aortic bifurcation. An iliac vein local thermodilution catheter was inserted into the right and left iliac veins (6). These animals were not subsequently treated with guanethidine.

In all animals, cannulation of the central ear artery and right atrium, and insertion of a tracheotomy tube were performed on the day of the experiment under local lidocaine anesthesia, as described previously (4).

Cardiorespiratory Measurements.—Cardiac output was determined from thermodilution curves recorded from the upper abdominal aorta after rapid injection into the right atrium of 0.46 ml of 5.5% dextrose in distilled water at room temperature, using the formulas and corrections described previously (4, 5). No significant difference was observed in mean estimates of cardiac output during simultaneous comparisons obtained in rabbits inhaling air and during hypoxia of the thermodilution and dye dilution methods (4), and of thermodilution and Fick methods (9). In each instance, the regression line did not differ significantly from the line of identity, and the standard deviation was ±7% of the mean thermodilution estimate. Portal, renal, and common iliac vein blood flows were determined by local thermodilution as described previously (5, 6). The accuracy of local thermodilution was estimated in a circulation model in vessels of the same size as the various veins in the rabbit, and in vivo in the rabbit's renal vein, where the thermodilution estimates were compared to the PAH clearance from the same kidney (5). The estimates by thermodilution and the independent flow methods did not differ significantly, and the standard deviation was ±8% of the mean thermodilution estimate (5). Skin blood flow was estimated by a thermal conductivity method, calibrated in separate perfusion experiments relating heat flow to cutaneous blood flow as described previously (14, 16); heat flow disks were applied to two sites on the hind limb and to the ear (15). Muscle blood flow was estimated as the difference between simultaneously recorded common iliac vein flow and total hind limb skin blood flow (6); ankle skin blood flow was considered representative of hind limb skin blood flow. Bone blood flow was neglected, as was also the venous flow from the pelvic contents, which in the rabbit drains mostly into the tail vein (6).

Mean ear artery pressure and right atrial pressures were recorded using Statham P 23 AC strain gauges, and the heart rate was obtained from the arterial pressure record. Total peripheral resistance was calculated as the ratio (ear artery pressure - right atrial pressure)/cardiac output. In the peripheral beds, vascular "resistance" was calculated as ear artery pressure/peripheral blood flow, neglecting venous pressure, since pressure recording through the slit of the injection lumen of the local thermodilution catheter proved unreliable because the slit usually closed between injections.

The animals breathed room air through a low resistance respiratory flap valve during resting.
and recovery periods (4, 17), and a freshly prepared low O<sub>2</sub>-N<sub>2</sub> mixture (7.5 to 8% O<sub>2</sub>) was administered during the test period from a light latex balloon (17). In previous experiments, the cardiorespiratory variables of the animals remained stable throughout the experiment, when breathing room air through the valve during resting and recovery periods and 21% O<sub>2</sub> from an air cylinder from the valve-balloon assembly during the test period (17). Respiratory rate was measured by counting the respiratory movements and minute ventilation (liters/min, ATPS) by collecting the expired gas. Arterial Po<sub>2</sub>, Pco<sub>2</sub>, and pH were measured with a (Instrumentation Laboratory Inc. Model 113), blood gas analyzer and pH meter using 1.5 ml of blood per sample, collected anaerobically.

**Experimental Procedures.**—After the initial cannulation procedures, each animal rested without restraint for 1 hour inside a small rabbit box. The experiment consisted of a resting period of 16 minutes inhaling room air, during which 5 sets of cardiorespiratory measurements were made (cardiac output or the various peripheral blood flows, arterial and right atrial pressures, heart rate, respiratory rate, and minute volume). This was followed by a sudden change in inspired gas concentration to 7.5 to 8% O<sub>2</sub> and 17 sets of cardiorespiratory measurements were made, followed by a recovery period inhaling room air, with 4 sets of recovery measurements made during the next 13 minutes. One set of measurements of arterial Po<sub>2</sub>, Pco<sub>2</sub> and pH was obtained while the rabbits breathed room air, and two measurements were obtained 12 and 32 minutes after the start of hypoxia (Table 1).

The animals tolerated the severe hypoxia well. They usually were somewhat restless for the first 15 to 30 seconds after the start of hypoxia but quickly settled down and remained quiet and in good condition and could run about in the laboratory immediately following the experiment. The autonomically de-efferented animals appeared more affected by the stress than the normal animals and moved little in the box but did not appear to lose consciousness. They also recovered rapidly and were alert and in good condition when placed on the laboratory floor at the end of the experiment.

There were five series of experiments:

**Series 1:** Changes in total peripheral resistance and heart rate during hypoxia were studied in groups of normal and autonomically de-efferented rabbits to assess net autonomic effects with only one experiment performed in each animal (Fig. 1). Similar observations were also made in a group of adrenalectomized animals.

**Series 2:** Changes in total peripheral resistance and heart rate were studied in the same animal before and after administration of atropine (Fig. 2), after an initial dose of 3 mg of atropine sulfate iv, given 10 minutes before the test, followed by 0.2 mg/min iv by continuous infusion. The high dose was necessary because of the high atropinase concentration in the rabbit, was without toxic effects, and blocked heart rate changes after electrical stimulation of the vagus (3, 18).

**Series 3:** Vagal and cardiac sympathetic component of the cardiac chronotropic effects were assessed in three animals (Fig. 3), by studying each without drugs, while atropinized, and while treated with propranolol, 0.3 mg/kg/15 min iv, which blocks the tachycardia normally produced by rapidly injecting 4 μg of isoprenaline sulfate iv (19). The order of treatment was arranged in the form of a Latin square (20). The 90 minutes of recovery between tests was adequate to avoid residual effects from propranolol and atropine (3, 18, 19).

**Series 4:** Peripheral vascular resistance was determined simultaneously in the portal, renal, gastrointestinal, and mesenteric arteries of five groups of normal and autonomically de-efferented rabbits, at the start of hypoxia and 32 minutes after the start of hypoxia.

**TABLE 1**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Air</th>
<th>12 min</th>
<th>32 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PaO&lt;sub&gt;2&lt;/sub&gt; (mm Hg)</td>
<td>95 ± 2.4</td>
<td>31 ± 1.6</td>
<td>30 ± 0.9</td>
</tr>
<tr>
<td>Pco&lt;sub&gt;2&lt;/sub&gt; (mm Hg)</td>
<td>36 ± 1.8</td>
<td>18 ± 1.6</td>
<td>16 ± 1.2</td>
</tr>
<tr>
<td>pH</td>
<td>7.46 ± 0.03</td>
<td>7.60 ± 0.02</td>
<td>7.59 ± 0.04</td>
</tr>
<tr>
<td>De-efferented</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PaO&lt;sub&gt;2&lt;/sub&gt; (mm Hg)</td>
<td>90 ± 1.7</td>
<td>31 ± 0.7</td>
<td>32 ± 1.1</td>
</tr>
<tr>
<td>Pco&lt;sub&gt;2&lt;/sub&gt; (mm Hg)</td>
<td>38 ± 1.8</td>
<td>20 ± 2.0</td>
<td>18 ± 1.0</td>
</tr>
<tr>
<td>pH</td>
<td>7.48 ± 0.03</td>
<td>7.68 ± 0.02</td>
<td>7.59 ± 0.07</td>
</tr>
</tbody>
</table>

Values are means ± SEM.
muscle, and skin beds in normal and autonomically de-efferented animals, to determine the net autonomic peripheral effects (Fig. 4).

Series 5: Vascular resistance was determined in muscle and skin beds in adrenalectomized animals with one limb normally innervated, and one limb sympathectomized (Figs. 5 and 6).

Statistical Methods.—In each experiment the 26 measurements of each variable were grouped into the 10 larger time intervals shown in Figure 1, the timing being identical in each animal. In each animal two or three measurements of each variable were averaged per time interval, except for the first interval after induction of hypoxia, when only one set of measurements was obtained. The mean absolute value of each variable was determined at every time interval by averaging the results obtained from all the animals in the group. To facilitate comparisons the average change at each time interval during hypoxia and recovery has been expressed as a percentage of the mean resting value of the group (Fig. 1).

Absolute resting values are given in Figures 1 and 4, and in legends to Figures 2 and 3. The standard error of the mean at a single time interval was calculated from analysis of variance after subtracting between-time-intervals, and between-animals from the total sums of squares as (error mean square/n), where n is the number of animals in the group (20). Significance of differences between time intervals was determined by variance ratio and t-test. For a comparison of the difference in response at any given time interval between two groups with SE1 and SE2 of the mean at a single time interval and n1 and n2 animals respectively, the standard error is \( (SE_1^2 + SE_2^2)^{1/2} \), with \((n_1 + n_2 - 2)\) degrees of freedom. For assessing the significance of the difference between two groups over more than a single time interval, the SE = \( (SE_1^2/ N + SE_2^2/ N)^{1/2} \), where N is the number of longer time intervals and \((n_1 + n_2 - 2)\) is the degrees of freedom. Because of the small numbers in the groups of series 2, in which the effects of hypoxia were studied before and after atropine in the same animal, the degrees of freedom were considered as though the two series were separate—as \((2n - 2)\), rather than \((n - 1)\).

Assumptions and Limitations.—The thermodilution techniques for measuring cardiac output and venous blood flow have about the same accuracy as other mean blood flow methods, with a standard deviation of 7 to 8% of the mean value. The errors in estimation appear to be normally distributed \((4, 5, 9)\). However the partitioning of common iliac vein flow into hindlimb skin and muscle blood flow introduces systematic errors into the estimates of each of these two variables owing to (1) neglect of bone flow and that component of pelvic venous drainage which does not enter the tail vein \((6)\); (2) differences between animals and limb sites in relation between cutaneous blood flow and heat flow \((14-16)\); (3) inadequacies in the sampling by the heat disks to describe variation in blood flow in different regions of hindlimb skin due to the hairgrowth cycle \((21)\). Factor 1 is probably small \((6)\), but factors 2 and 3, which are probably partly interrelated, can produce a difference between animals of the order of 30% of estimated skin blood flow for a given heat flow, and will thus also result in differences in estimated muscle blood flow. In terms of estimates of absolute flow, these errors are not large, since the total estimated hindlimb skin flow is 9 ml/min, while the estimated muscle blood flow is 20 ml/min. Furthermore these errors appear to be normally distributed in a group of rabbits \((6, 14-16)\), and the mean results of portal, renal, muscle, and skin blood flow, when expressed as a fraction of the cardiac output, are almost identical with corresponding values observed in other species \((5, 6)\). This suggests that despite the systematic error in muscle and skin blood flows in individual animals, the results from a whole group of rabbits provide reasonable estimates of absolute blood flow. In the present study accurate assessment in the same group of animals of changes during hypoxia from the resting value is required, and this is relatively unaffected by systematic errors in individual animals. Using multiple measurements and studying the responses of groups of 3 to 6 animals, precision in estimating changes has been increased considerably. The preparation is stable, since during inhalation of 21% O2 during a period equal to the length of a whole experiment, changes in the different circulatory variables are minimal, the standard error of the mean cardiac output at a single time interval being \(\pm 2.6%\), and the SE of the mean resting value being \(\pm 1.7%\) (6 animals) \((17)\). An approximately similar degree of accuracy has been obtained in estimating changes in regional blood flow including skin flow \((5, 6, 14-16)\).

The response pattern of the different variables during hypoxia has been examined on a number of occasions in different groups of 3 to 6 unanesthetized normal and de-efferented rabbits of the same strain, sex, approximate age and weight as in the present series, and virtually identical effects have been obtained in a given preparation on each occasion \((2, 17, 19, 22)\). The assumption made in the present study that a group of 3 to 6 similarly treated rabbits gives a representative response thus appears to be reasonable (Figs. 1 and 2). The present analysis of the different autonomic effects has, however, the general limitation that the conclusions are
based on an indirect reconstruction of the "normal" response derived from a number of different experimental groups. Furthermore, grouping the results into relatively long time intervals permits analysis of only gradual changes in response.

The estimation of local effects from the response of autonomically de-efferented animals depends in part on the completeness of inactivation of the autonomic effectors in these animals. The steroid maintenance on fixed glucocorticoid and mineralocorticoid replacement appears to be complete; the animals die 2 to 3 days after withdrawal of steroid therapy (7, 9). We have assumed that the steroids do not produce abnormalities in cardiovascular responsiveness, since animals subjected to adrenalectomy alone (without guanethidine treatment) have closely similar reflex changes in heart rate and total peripheral resistance during hypoxia as normal animals (23, 24). The dose of guanethidine used completely blocks sympathetic nerve transmission at constrictor and cardiac nerve endings, preventing release of neurotransmitter onto the alpha- and beta-receptors respectively. Guanethidine appears not to interfere with carotid body function in the rabbit in view of the approximately normal respiratory response to hypoxia of de-efferented animals (2, 15, 23). Its effect on carotid body catecholamines in the rabbit is not known, but in the present dose the drug does not deplete the catecholamine stores of the adrenal medulla (11-13, 23, 24). The adrenalectomized guanethidine-treated animals are in good condition, active and alert, the main side effect of guanethidine being slight diarrhea, ptosis of the eyelids, and some nasal stuffiness. We have assumed that the main effect of atropine is to block vagal efferents (2), that its effect on chemoreceptor discharge can be neglected in the doses used (25), and that when used by itself in series 2 and 3 its recently demonstrated effect on sympathetic ganglion transmission can probably be neglected in the presence of a normally functioning nicotinic component (26). Apart from blocking cardiac and peripheral beta-receptors, propranolol produces myocardial depression (27), which appears to be less marked in unanesthetized rabbits than in anesthetized (6, 28), but may have contributed to the slightly more marked bradycardia in Figure 3.

Results

**ESTIMATION OF NET AUTONOMIC EFFECTS**

Figure 1A shows that the absolute resting values of heart rate, arterial pressure, cardiac output, and total peripheral resistance of the de-efferented animals were about 90% of the corresponding values of normal animals, only the differences in heart rate being statistically significant \( P < 0.05 \) [15]). Time-autonomic heart rate effect curves (vagal + sympathetic
chronotropic effects) were constructed by superimposing the resting heart rate values of the normal and de-efferented animals, and expressing the changes in each preparation as the percent of its mean resting value (Fig. 1B, top graph). We defined the change in autonomic heart rate effect at each time interval as the difference in percent response between the two preparations (autonomic + local effect in normal animals minus local effect in de-efferented animals) and plotted the time-autonomic heart rate effect curve by joining the differences at successive time intervals (Fig. 1B, bottom graph). In the same way, time-autonomic peripheral resistance effect curves were constructed by superimposing the resting total peripheral resistance values of the two preparations, expressing the respective changes at each time interval during hypoxia as the percent of the resting value (Fig. 1C, top graph), and joining the successive mean differences (Fig. 1C, bottom graph).

The standard error of the mean autonomic effect at a single time interval, $SE_{aut} = (SE_{normal}^2 + SE_{de-effere}^2)^{1/2}$, where the values in brackets are the standard error of mean heart rate or total peripheral resistance at a single time interval in normal and de-efferented animals respectively. For assessing the statistical significance of the changes in autonomic effects, the number of degrees of freedom for $n_1$ normal and $n_2$ de-efferented animals is $(n_1 + n_2 - 2)$. When comparing the differences between autonomic effects of two groups of animals ($n_1$ normal and $n_a$ adrenalectomized animals) the degrees of freedom are $(n_1 + n_a - 2)$, and the de-efferented animals contribute only to the calculation of $SE_{aut}$ of each group.

**RESPONSE TO HYPOXIA AND NET AUTONOMIC EFFECT CURVES**

The response to hypoxia ($P_{ao_2}$ 30 mm Hg; Table 1) in normal animals included marked initial bradycardia; a transient rise in blood pressure, falling slightly below resting during the latter part of hypoxia; an initial fall in cardiac output and transient rise in total peripheral resistance (Figs. 1 and 2; [17, 22]). In autonomically de-efferented animals a similar change in blood gas composition

![Figure 2](http://circres.ahajournals.org/)

**FIGURE 2**

A: Mean changes during hypoxia expressed as a percent of the resting value in heart rate (H.R.), arterial pressure (B.P.), cardiac output (C.O.), right atrial pressure (R.A.P.), and total peripheral resistance (T.P.R.) in 3 animals studied under normal conditions without drugs (N), and while atropinized (At). Absolute mean resting values are listed for each variable in the order: no drug then with atropine; H.R. = 267/min, 300/min; B.P. = 100 mm Hg, 95 mm Hg; C.O. = 506 ml/min, 475 ml/min; R.A.P. = 0 mm Hg, –1.6 mm Hg. B: Time course in autonomic heart rate effects: Net (N, solid circles), i.e., normal-de-efferented heart rates; sympathoadrenal (S-A) open circles, i.e., atropinized-de-efferented heart rates; vagal (V, dotted line), i.e., (N) – (S-A) heart rates. C: Time-autonomic total peripheral resistance effect curves without drugs (solid line) and after atropine (broken line).
resulted in little change in heart rate; the cardiac output rose initially and the total peripheral resistance fell to an approximate plateau value of about 70% of resting (Fig. 1). The respiratory response of the autonomically de-efferented animals was normal, as described previously (2, 10).

The autonomic heart rate effect at \( \text{Pao}_2 \) 30 mm Hg consisted of maximum cardiac slowing at the beginning of hypoxia, diminishing with time (Figs. 1B and 2B). In another group of rabbits subjected to more severe hypoxia (mean \( \text{Pao}_2 \) 27 mm Hg) the initial autonomic cardiac slowing disappeared more rapidly, and the autonomic effect toward the latter part of hypoxia consisted of a rise in heart rate (Fig. 3).

The autonomic total peripheral resistance effect at \( \text{Pao}_2 \) 30 mm Hg rose considerably at the start of hypoxia and then declined in an approximately exponential manner (Figs. 1C and 2C). During the latter part of hypoxia it was still significantly increased above resting (\( P = 0.01 \), based on the results of 7 normal animals in Figs. 1 and 2).

**TABLE 2**

Mean Arterial Pressure at Various Times after Start of Hypoxia and during Recovery as Percent of Preceding Mean Resting Value

<table>
<thead>
<tr>
<th></th>
<th>Rest*</th>
<th>1 min</th>
<th>4 min</th>
<th>9 min</th>
<th>17 min</th>
<th>27 min</th>
<th>39 min</th>
<th>Recovery</th>
<th>±SE$^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>100(91)</td>
<td>106</td>
<td>101</td>
<td>98</td>
<td>99</td>
<td>94</td>
<td>94</td>
<td>102</td>
<td>1.9</td>
</tr>
<tr>
<td>Atropine</td>
<td>100(85)</td>
<td>122</td>
<td>117</td>
<td>110</td>
<td>102</td>
<td>94</td>
<td>95</td>
<td>100</td>
<td>2.7</td>
</tr>
<tr>
<td>Propranolol</td>
<td>100(8)</td>
<td>102</td>
<td>101</td>
<td>101</td>
<td>100</td>
<td>98</td>
<td>95</td>
<td>100</td>
<td>2.4</td>
</tr>
</tbody>
</table>

Each of 3 rabbits was studied without drugs (normal), while atropinized, and after propranolol. The mean \( \text{Pao}_2 \) was 27 mm Hg with each treatment.

*Value in parentheses is the resting arterial pressure in mm Hg. $^*$Time in minutes is the time after the start of hypoxia (cf. Fig. 1). $^*$Standard error of mean observations at a single time interval calculated from analysis of variance.

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SELECTIVE AUTONOMIC BLOCK

Effect of Atropine.—After atropinization the vagal efferents are blocked, and the difference between atropinized and de-efferented animals is a function of the reflex changes in sympathoadrenal effects. Whether the sympathoadrenal effects in the atropinized animal are the same as the true sympathoadrenal effects in the normal animal will depend on whether the hemodynamic differences between the normal and atropinized groups are sufficient to alter the profile of stimulation of the peripheral receptors, thereby changing the output through the unblocked autonomic effectors.

Three rabbits were studied before and after atropinization (Fig. 2). After atropine the fall in heart rate during the early part of hypoxia was smaller than before, and the autonomic heart rate effect was biphasic, with the initial reflex slowing being followed by reflex tachycardia toward the latter part of hypoxia. After atropine the rise in arterial pressure was more marked than normal during the first 20 minutes of hypoxia \((P = 0.02)\), and the autonomic total peripheral resistance effect was less marked than normal during the first 4 to 6 minutes of hypoxia \((P < 0.05)\).

The greater rise in blood pressure during the early part of hypoxia after atropine would be expected to distort the true sympathoadrenal effects. This was assessed from the heart rate and blood pressure changes in another group of three rabbits, each studied at mean \(Pao_2\) 27 mm Hg (range 25 to 29 mm Hg) (Fig. 3, Table 2) without drugs, after atropine, and after propranolol. The heart rate changes from a group of de-efferented animals are also shown in Figure 3. After atropine the blood pressure response to hypoxia was again exaggerated in this series, but after propranolol it was the same as without any drug (Table 2). Sympathoadrenal effects on heart rate were estimated (1) from the difference in response between normal and propranolol-treated animals (sympathoadrenal + vagal + local minus vagal + local effects) in the presence of a normal blood pressure response in each group; (2) from the difference between atropinized and de-efferented animals (sympathoadrenal + local minus local effects) in the presence of an exaggerated pressor response in the atropinized animal. The estimated magnitude of the early sympathoadrenal effect on heart rate was the same in estimates 1 and 2 (Fig. 3, middle panel), but its duration was slightly more prolonged in estimate 2 \((P = 0.07)\), obtained during the exaggerated pressor response. The vagal effects on heart rate estimated from the difference in changes between normal and atropinized animals (sympathoadrenal + vagal + local minus sympathoadrenal + local effects) were correspondingly of shorter duration than

![Figure 4](http://circres.ahajournals.org/figures/cirres24f4.jpg)
the estimates obtained from the difference between propranolol-treated and de-efferented animals (vagal + local minus local effects) (Fig. 3, right panel). The results are consistent with a small secondary reflex effect due to the greater pressor response after atropine through the unblocked cardiac effector pathway.

Effect of Adrenalectomy.—The circulatory response to hypoxia in the rabbit is little modified by adrenalectomy (23, 24). The autonomic heart rate and total peripheral resistance effects at \( P_{\text{ao}} \) 27 mm Hg were not significantly different between a group of normal and a group of adrenalectomized animals (Table 3).

PERIPHERAL AUTONOMIC EFFECTS

Net Effects.—The resting absolute peripheral blood flow values of normal and de-efferented animals were closely similar (Fig. 4 [15]). In normal animals there was a marked rise during hypoxia in vascular resistance in portal and renal beds, changes in muscle resistance were relatively small, and in the skin there was moderate reduction in resistance in hindlimb and a marked fall in the ear. In de-efferented animals reduction in resistance was markedly non-uniform, with the greatest fall observed in portal bed, and the least in muscle (6, 14, 15). The resting vascular resistance values of normal and de-efferented animals were superimposed (Fig. 1), the response during hypoxia of each group expressed as the percent of resting at each time interval, and time-autonomic peripheral resistance effect curves constructed from sequential differences in response between the groups at each time interval.

In the portal and renal beds the autonomic effect on peripheral resistance increased sharply soon after the onset of hypoxia, subsiding to an approximately steady plateau level of about 50% to 60% of the early maximum effect, and returning to resting soon after the onset of hypoxia. In muscle the estimated changes in autonomic effect were small, while in skin the autonomic effect on vascular resistance fell below resting during hypoxia, i.e., the reduction in vascular resistance in normal animals was greater than in the de-efferented. The fall was more marked in ear skin than in hindlimb.

Muscle.—Chalmers et al. (6) have shown previously that the opposing influences during hypoxia of effects on alpha-receptors mediated through the constrictor nerves, and dilator effects on beta-receptors exerted by small amounts of epinephrine (virtually the rabbit's
TABLE 3
Mean Change in Autonomic Effects on Heart Rate and Total Peripheral Resistance in Five Normal and Five Adrenalectomized Rabbits Maintained on Steroid Therapy

<table>
<thead>
<tr>
<th></th>
<th>1 min</th>
<th>4 min</th>
<th>9 min</th>
<th>17 min</th>
<th>27 min</th>
<th>39 min</th>
<th>±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heart Rate (beats/min)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>-22</td>
<td>-18</td>
<td>-6</td>
<td>+4</td>
<td>+9</td>
<td>+5</td>
<td>7.0</td>
</tr>
<tr>
<td>Adrenalectomized</td>
<td>-22</td>
<td>-19</td>
<td>-16</td>
<td>0</td>
<td>+14</td>
<td>+17</td>
<td>4.0</td>
</tr>
<tr>
<td><strong>Total Peripheral Resistance (units)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>+75</td>
<td>+52</td>
<td>+34</td>
<td>+27</td>
<td>+39</td>
<td>+42</td>
<td>11.0</td>
</tr>
<tr>
<td>Adrenalectomized</td>
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<td>+50</td>
<td>+34</td>
<td>+19</td>
<td>+29</td>
<td>+38</td>
<td>12.0</td>
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</table>

Each rabbit was studied at a mean Po₂ of 27 mm Hg. Minutes is time from the start of hypoxia (cf. Fig. 1). se is standard error of mean at a single time determined by analysis of variance as described in text.

sole adrenal medullary hormone [29]), account for the absence of marked changes in muscle peripheral resistance. Dilator effects through cholinergic vasodilators appear to be absent in the rabbit (6, 30). Figure 5 illustrates the changes in muscle blood flow and vascular resistance from another group of normal rabbits and from a group of adrenalectomized rabbits with one limb innervated and the other surgically sympathectomized.

The neural autonomic effects were estimated from the differences in simultaneously estimated resistance in innervated and sympathectomized limbs of the adrenalectomized animals (neural + local minus local effects). After a latency of about 5 minutes, the neural autonomic effect increased progressively (Fig. 5C, bottom graph). The effects of epinephrine were estimated from the difference in resistance between normal and adrenalectomized
animals with innervated limbs (neural + adrenal + local minus neural + local effects). Epinephrine produced reduction in autonomic resistance effect, which began after a latency of about 5 minutes and became progressively more pronounced in the course of hypoxia. The dose-response curve relating epinephrine concentration to muscle resistance is biphasic (Fig. 5, middle graph; 6, 31). The gradually increasing autonomic dilator effect will result from small concentrations of epinephrine rising slowly from zero (29), and the autonomic dilator response can thus be equated approximately with adrenal medullary output. The blood pressure response during hypoxia was different in adrenalectomized animals (mean, 92% of resting) than in normal animals (mean, 101% of resting; $P = 0.05$), so that slight distortion in magnitude and time course of the estimated autonomic effects may have occurred.

Skin.—In adrenalectomized animals the vascular resistance fell to a significantly greater degree in the innervated hindlimb than in the sympathectomized side (mean difference during hypoxia, 11 ± 2.1 (SE) %; 0.05 > $P > 0.02$) (Fig. 6). Since cholinergic effects on the rabbit's skin vessels (32) can probably be excluded during hypoxia (14), the significantly greater fall in resistance on the innervated side than on the sympathectomized side is due to inhibition of resting sympathetic vasoconstrictor tone in this bed.

**COMPOSITE PATTERN OF AUTONOMIC AND RESPIRATORY EFFECTS**

Each effect in Figure 7 has been scaled in terms of the greatest positive or negative change ($± 100\%$) attained during or immediately after hypoxia. Changes in epinephrine secretion (estimated from the vascular response in muscle) have been expressed as a positive effect, since the resting epinephrine secretion is close to zero (29). No corrections have been applied to the estimated autonomic effects in experiments involving selective block (Fig. 3).

The changes in net autonomic total peripheral resistance effect indicate a preponderance of constrictor over dilator effects, which is most marked during the early part of hypoxia, though even after 44 minutes there is still significant net constriction. We subdivided the peripheral autonomic effector response into (1) an early component, becoming most marked soon after the onset of hypoxia and then decaying toward the resting value at the same rate as the initial vagal effect, with a time constant (the time to change from 100%
to 37% of the initial effect) of about 5 minutes; (2) a late component, having a time course similar to the estimated neural autonomic effect in muscle or to the secretion of epinephrine, and rising gradually after a latency of about 5 minutes toward a plateau near the end of hypoxia with a time constant (the time to change from 0% to 63% of maximum) of about 15 minutes (Fig. 8, broken line). The four different autonomic effector patterns to the heart and periphery can be considered to result from different combinations of the above components: (1) The increase in autonomic resistance effect in portal and renal beds, with a maximum reached soon after the onset of hypoxia and a plateau of 50% to 70% of this value, can be considered as the sum of the early and late components (Fig. 8). (2) The fall in cutaneous autonomic resistance effect has a time course which is the approximate mirror image of the above response and can be considered as the sum of negative early and late components; the time course of the vagal effects on heart rate is also similar, but the plateau is only about 30% of the initial effect (Fig. 7). (3) The biphasic sympathoadrenal effect on heart rate can be considered as the sum of a negative early and a positive late effect (Fig. 8). (4) The neural constrictor effect in muscle, and the estimated epinephrine secretion have only a late component.

Respiration.—The respiratory frequency rose to a maximum soon after the onset of hypoxia, and then declined toward resting at a rate slightly slower than the early component of the autonomic response (Fig. 7). An immediate rise in tidal volume was followed by a fall and a slower secondary rise toward a plateau near the end of hypoxia. The time course of the early and late components of this response were both faster than the corresponding cardiovascular autonomic components. The minute ventilation (V_T x f), also increased rapidly to a maximum, subsiding to a well-maintained plateau at about 70% of the initial maximum effect.

Discussion

ESTIMATION OF AUTONOMIC EFFECTS

The major assumption in estimating the autonomic effects is that the local (nonautonomic) effects during hypoxia are approximately the same in normal and de-efferented animals so that the autonomic effects can be simply estimated as the difference between normal and de-efferented responses. Factors which could contribute to differences in the local effects between the two groups include...
changes in vessel sensitivity due to the de-efferentation procedure, higher constrictor tone in normal animals, and differences in the transmural pressures between preparations during hypoxia.

The de-efferentation procedure probably does not alter vessel or cardiac pacemaker sensitivity to hypoxia, since in animals with section of the carotid sinus and aortic nerves (where the neural control loop has been broken at the input but the autonomic effectors remain structurally intact), the heart rate also changes little during hypoxia (17, 18, 22), and the time course of the peripheral dilator effects is the same as in de-efferented animals, while their slightly greater magnitude can be accounted for by differences in resting vessel tone and in $P_{aco_2}$ (due to abolition of the respiratory response after section of the carotid sinus and aortic nerves [15]). Guyton and his colleagues have suggested that hypoxia acts directly on the arteriolar smooth muscle cells rather than through the intermediate liberation of tissue metabolites, since they found similar changes in vascular conductance in vessels completely isolated from the surrounding tissues, as in the perfused whole organ (33, 34). Their model implies that the local dilator response in arterial hypoxia is a function of arterial $P_{o_2}$ and relatively independent of whole organ blood flow and vasomotor tone. The transmural pressure during hypoxia differs in normal and de-efferented animals, since in the former the arterial pressure changes only a slight amount, while in the latter it falls from 85 mm Hg resting to approximately 60 mm Hg during hypoxia. Johnson (35) has recently determined the magnitude of the dilator response during changes in transmural pressure in the arterioles of the cat's mesentery, and has found that sudden reduction in arterial pressure of the above magnitude results in a small transient arteriolar dilatation, but produces little permanent effect during equilibrium. Since the fall in arterial pressure in the de-efferented animal is gradual (Fig. 1), little dilator effect would be expected to result from the transmural pressure change, assuming that arterioles of other beds behaved in the same way as the cat's mesentery. The circulation in the de-efferented animal is relatively stable during the latter part of hypoxia (Figs. 1 and 4), suggesting that any progressive accumulation of metabolites occurs quite slowly. The circulatory effects are rapidly reversible, indicating that hypoxia produces no permanent damage. The assumption of similarity in local effects in normal and de-efferented animals thus appears to be a reasonable first approximation. The changes in autonomic effects will only be qualitatively related to autonomic effector activity, since their relationship is nonlinear (31, 36). Since the time intervals for grouping the results are relatively long, the autonomic effects can be estimated from successive differences in the mean values of the normal and de-efferented preparations, but only gradually changing autonomic effects can be studied.

The assessment of net autonomic effects from the responses of open and closed-loop neural control systems is probably associated with little distortion in the time course, since no rapid compensating systems are available to the de-efferented animal. On the other hand after selective block of a single autonomic effector there is the theoretical likelihood of distortion of both the magnitude and time course of the autonomic effects (37). The absence of significant change in the magnitude of the cardiac sympathoadrenal response after atropine may reflect a nonlinear response of the cardiac sympathetic to the baroreceptor reflex (38). The degree of distortion of the time course of the cardiac autonomic effects after atropine is relatively small, and there is probably also relatively little error in the assessment of the time course of the neural constrictor effects in muscle obtained in adrenalectomized animals, since a similar slow rise in neural constrictor effect has been observed in normal animals with intact adrenals, in which the effects of epinephrine on beta-receptors have been blocked by propranolol (6).

**AUTONOMIC EFFECTOR RESPONSE DURING HYPOXIA**

The rabbit has a smaller respiratory re-
response to hypoxia than man or the dog, but has a more marked autonomic circulatory response. The latter has some of the features of diving animals subjected to submersion asphyxia (17, 39-41). Its ability to tolerate the severe and prolonged reduction in \( \text{Pao}_2 \) without apparent ill effect is probably related to these reflex circulatory adjustments. The response may thus be regarded as a model of a “successful” cardiovascular adaptation to severe stress.

The early autonomic effects are entirely neurally mediated, and include the increased portal and renal constrictor effects, the autonomic slowing of heart rate due to increased vagal and decreased cardiac sympathetic activity, and the inhibition of neural constrictor tone in skin. The changes in net autonomic total peripheral resistance effect suggest a considerable preponderance in constrictor over dilator effects at this time. The splanchnic vasoconstriction will raise the arterial pressure and increase cerebral and coronary perfusion despite reduction in cardiac output, while the reflex bradycardia will contribute to the reduction in cardiac work. We can only speculate on the significance of the inhibition of cutaneous constrictor tone. It could have adaptive advantages by raising skin temperature and perhaps prevent a fully fledged “diving” response, which is potentiated by cold (40).

Both neural and adrenal medullary hormones contribute to the late phase of hypoxia. The small quantities of epinephrine secreted in this type of hypoxia (23, 24) probably enhance only slightly the neural constrictor effects on alpha-receptors in portal and renal bed. In muscle, epinephrine, through its effects on beta-receptors, inhibits neural constriction and maintains normal blood flow. The increased cardiac sympathetic effect produces a reflex rise in heart rate late in hypoxia, and probably contributes to the increase in cardiac output. At a time when visceral blood flow is still below resting, and muscle blood flow is normal, the rise in cardiac output will assist cerebral and coronary perfusion (15). The gradual fall in arterial pressure after the first few minutes of hypoxia (Fig. 1) suggests that local dilator factors resulting from prolonged hypoxia may eventually get the upper hand, but the ability of the animals to resume normal activity at the end of the experiment suggests that the circulatory control system has up to this point effectively compensated for the stress.

The fact that all the autonomic processes from ear to hindlimb can be described in terms of the same early and late autonomic components suggests that the autonomic effector patterns are determined mostly by ponto-medullary and suprabulbar, rather than by spinal mechanisms (42). The significance of the different combinations of signs of the early and late components which produce the four distinctive effector patterns has not been analyzed in these experiments. However, a recent neural analysis has shown that the early reflex slowing of heart rate (due to increased vagal and reduced cardiac sympathetic activity) requires activation of suprabulbar centers of the central nervous system, while the late reflex tachycardia is mediated through bulbar centers (42). The different combinations of signs of the various components in the autonomic effects on other target organs may also reflect differences in activation of bulbar and suprabulbar centers.

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The early and late components probably arise in response to different peripheral stimuli in the course of severe hypoxia. In the rabbit the primary contribution to the increased autonomic effects is through the arterial chemoreceptors and baroreceptors (17, 19, 25). In this species, as in the dog, vagal afferents signalling pulmonary inflation also play a role by inhibiting the reflex effects (on heart rate and constrictor tone) of strong chemoreceptor stimulation, but this is probably more important during mild hypoxia before the animal has reached its maximum respiratory response (28, 43). Probably the arterial chemoreceptors are chiefly involved in the early autonomic effects, since perfusing them with hypoxic blood produces similar effects (44). The relation of the late component to the slow decline in arterial pressure...
suggests involvement of a blood pressure control mechanism. During the latter part of hypoxia there is probably some adaptation of the chemoreceptors in the normal animal, and the late component probably arises through the arterial baroreceptors acting in conjunction with the chemoreceptors (42). It seems unlikely that the late autonomic effects result from progressive cerebral ischemia due to the fall in blood pressure, since no increase in autonomic effects has been observed in animals with section of the carotid sinus and aortic nerves in which the arterial hypotension is much greater (15). Local cerebral vasoconstriction due to moderate hypocapnia (Table 1) is another possibility, but this is probably offset by the local cerebral dilator effects of the severe hypoxia (45).

The net autonomic effects on heart rate and total peripheral resistance both have dynamic characteristics similar to the lead-lag first-order negative feedback control system (37). Such a system responds to a step input with a finite jump, which subsequently decays to a steady-state level (Fig. 1). The responses of a number of biological receptors have the characteristics of a lead-lag system and are sensitive to the rate of change of the stimulus, as well as to its magnitude. The early autonomic component of the rabbit's response to hypoxia occurs at a time when the PaO₂ is decreasing rapidly, probably in response to a large increase in chemoreceptor activity, and before equilibrium has been reached at the periphery between autonomic and local effects. The late autonomic component appears to be evoked through a baroreceptor mechanism, probably in response to the local tissue effects of hypoxia. The early and late autonomic components together behave like a lead-lag negative feedback control system with rate sensitive and proportional control properties.

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Dynamic Characteristics of the Cardiovascular Autonomic Effects during Severe Arterial Hypoxia in the Unanesthetized Rabbit
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