EARLY AND LATE HIND-LIMB VASCULAR RESPONSES TO STIMULATION OF RECEPTORS IN THE NOSE OF THE RABBIT

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SUMMARY

1. In rabbits under pentobarbitone anaesthesia stimulation of the nasal mucous membrane with ether vapour causes apnoea, bradycardia and a rise in arterial blood pressure.

2. Simultaneous measurements of femoral arterial blood pressure and of femoral arterial or venous blood flow show that vascular resistance increases in both the intact and skinned hind limb in response to nasal stimulation. Evidence is presented to show that the increase in hind-limb vascular resistance is due to vasoconstriction which is reflex in nature.

3. The change in vascular resistance in the hind limb following nasal stimulation may be divided into two distinct phases. The primary (early) phase is mediated by the efferent sympathetic nerves to the limb whereas the secondary (late) phase is mediated by adrenal gland hormones.

4. The secondary phase of the hind-limb vascular response is invariably less pronounced than the primary phase, and with regard to the time course of the appearance of the two phases of the response it appears that following stimulation of the nose there is no mutual reinforcement of sympathetic neural and humoral influences on the hind-limb blood vessels.

5. The cardiovascular responses occur in the absence of changes in pulmonary ventilation.

INTRODUCTION

It is established that appropriate stimulation of certain receptors present in the nasal mucosa evokes striking responses in the respiratory and cardiovascular systems of many animal species (Kratschmer, 1870; Allen, 1928; Angell-James & Daly, 1969; White, McRitchie & Franklin, 1974). The

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responses may be elicited by a wide variety of agents acting on the nose or face (Allen, 1928; Andersen, 1966) and are of physiological interest not only because of their probable functional role in diving species (Bert, 1870; Richet, 1894; Scholander, 1940) but also because of their considerable clinical significance in man (Allison, 1974).

The principal effect observed in the respiratory system following stimulation of the nasal mucous membrane is a reduction in breathing or apnoea with, in some species, bronchodilatation (Tomori & Widdicombe, 1969; Allison, Clay, Hughes, Jones & Shevis, 1974). The cardiovascular response consists of bradycardia and variable blood pressure changes (Kratschmer, 1870; Brodie & Russell, 1900; Rall, Gilbert & Trump, 1945), a reduction in cardiac output, and vasoconstriction in skin, muscle, splanchnic and renal vascular beds. The vasoconstrictor response is mediated principally by the sympathetic adrenergic nerves (Angell-James & Daly, 1972; Powis, 1973; Allison 1974; White *et al.* 1974). It has been shown, however, that in the rabbit a significant increase in catecholamine secretion by the adrenal medulla occurs following nasal stimulation (Allison & Powis, 1971) and it is possible therefore that the vasomotor response to such stimulation represents the interaction of both nervous and humoral influences. The purpose of the present study was to evaluate the relative contributions of these two sympathetic mechanisms to the vascular effects reflexly evoked in the hind limb of the rabbit by stimulation of the nasal mucous membrane.

METHODS

New Zealand White rabbits of either sex, and of weight between 2·0 and 4·6 kg (mean 3·0 kg), were anaesthetized with a solution of pentobarbitone sodium (Nembutal, Abbott Laboratories Ltd, 40 mg/kg) injected slowly into a marginal ear vein. Subsequent doses of the anaesthetic agent (4 mg/kg) were administered as necessary through an indwelling polyethylene cannula in a femoral vein.

The urinary bladder was catheterized and continuously drained throughout all experiments.

Body temperature was maintained at $37 \pm 1\cdot5°$ C by a thermostatically controlled heated table and was monitored with a rectal thermometer. Heat and fluid losses were minimized by wrapping the animal in polyethylene sheeting and cotton wool.

Tidal volume and respiratory rate were recorded from a tracheostomy tube by means of a balanced spirometer and bag-in-box system (Bacon, Daly & Scott, 1962). In most experiments respiration was spontaneous throughout, the rabbits breathing a gas mixture of 45% O_2 and 55% N_2. In a few experiments positive pressure ventilation was employed at a rate of 28 c/min, the tidal volume being adjusted initially so that spontaneous respiratory efforts were just detectable.

Stimulation of the nasal mucous membrane. The head of the rabbit was elevated and inclined at an angle of 30° to the horizontal. This ensured that the venous drainage from the nose was not restricted (Angell-James & Daly, 1972). A cuffed tube similar to that described by Angell-James & Daly (1969) was inserted into the rostral end of the divided trachea and advanced so that its tip lay at the extreme

anterior end of the nasopharynx. In each test ether vapour from a Woulff bottle was blown through the tube and through the nose from the direction of the naso-pharynx for a period of two seconds and then eliminated from the nose by an immediate subsequent insufflation of room air. It has been shown that the reflex responses to stimulation of the nasal mucosa independent of the direction of gas flow through the nose (Ramos, 1960; Allison, 1974). In a few experiments the nasal passages were anaesthetized by the topical application of 10 ml. 2 % Lignocaine ('Lidothesin', Willows Francis Ltd), prior to stimulation with ether. This quantity of local anaesthetic did not have any measurable effect on resting blood pressure, heart rate, hind-limb blood flow or respiratory rate.

Measurement of vascular pressures. In every experiment arterial blood pressure was recorded from a femoral artery. In some experiments a mercury manometer was used and the pressures registered were displayed on smoked paper. In these experiments heart rate was measured from an electrocardiogram. In other experi-ments the arterial blood pressure was registered by means of a Statham P23Gb strain-gauge transducer calibrated before each experiment with a mercury mano-meter. Zero reference pressures were obtained post mortem with the cannula tip open to the air. Mean arterial blood pressure was extracted electronically by passing the amplified pulsatile blood pressure signal through a passive R-C network of time constant 1–2 sec. In these experiments heart rate was measured from a pulse fre-quency meter triggered by the pulsatile wave form from the arterial blood pressure transducer.

In a few experiments mean inferior vena caval pressure was registered by a Statham P23Gb strain gauge transducer connected to a catheter inserted through a femoral vein.

Measurement of hind-limb blood flow. Flow was measured in a femoral artery by means of photo-electric drop counter (Lindgren, 1958) and drop timer (Gaddum & Kwiatkowski, 1938). Blood from the cannulated central end of a femoral artery passed through the drop counter before entering the distal cannulated portion of the vessel.

The arterial perfusion pressure was measured from the tubing conveying blood from the drop counter to the femoral artery, and this tubing passed through a heated water-bath before entering the artery. The dead space of this extra corporeal circuit was 2·8 ml.

In some experiments blood flow was measured in the femoral vein by the method described by Powis (1974).

To define more precisely the vascular area under scrutiny, some experiments were conducted on the skinned hind limb. The skin was removed down to the ankle joint and a tourniquet applied at this level to exclude the paw circulation which is mainly skin (Daly & Robinson, 1968; Korner & Uther, 1969).

Calculation of hind-limb vascular resistance. Hind-limb vascular resistance was calculated according to the formula

$$\text{hind-limb vascular resistance} = \frac{\text{mean arterial blood pressure (mmHg)}}{\text{hind-limb blood flow (ml. min}^{-1})}$$

and expressed in resistance units representing the pressure necessary to force blood at 1 ml. min^{-1} through the hind-limb vascular beds.

The central venous pressure was measured in six tests in three animals. There was no significant change from the control value of $2 \cdot 5 \pm 0 \cdot 22$ cm H_2O ($0 \cdot 18 \pm 0 \cdot 016$ mmHg) during stimulation of the nasal mucous membrane, hence the errors introduced by ignoring the central venous pressure in the calculations of vascular resistance were considered to be negligible.

Determination of hind-limb vascular resistance under conditions of constant perfusion pressure. In some tests a compensating device similar to that described by Grayson & Johnson (1953) was used to maintain a constant perfusion pressure. This consisted of a reservoir of rabbit blood maintained at $37 \pm 1^\circ$ C and mixed by a magnetic stirring device. Pressure within the reservoir was monitored with a mercury mano-meter and maintained at the required level by varying the release of inflowing compressed air with an adjustable valve. The apparatus was connected to a side arm of the femoral arterial cannula; any change in femoral arterial pressure led to a flow of blood from the animal to the reservoir or vice versa, consequently mini-mizing any alterations in the mean perfusion pressure of the limb.

In a further series of tests the perfusion pressure of the hind limb was kept at a constant level by means of a manually operated device which permitted the tubing conveying blood to the hind limb to be compressed to a variable degree. Mercury manometers proximal and distal to this apparatus recorded the systemic and hind-limb perfusion pressures respectively, and the hind-limb vascular bed was protected from any increase in systemic arterial pressure occurring during nasal stimulation by appropriate adjustment of the attenuation device.

Statistical analysis. All results are expressed as mean values ± 1 s.e. of the mean. Where appropriate Student's *t* test was used to evaluate the difference between two means of grouped values.

RESULTS

Stimulation of the nasal mucous membrane with ether in the spon-taneously breathing rabbit caused an immediate period of apnoea in the expiratory position, bradycardia, a rise in mean arterial blood pressure (MABP) and a reduction in hind-limb blood flow (Fig. 1).

The mean duration of the apnoea in thirty tests (twelve animals) was 13 ± 3.5 sec and the mean fall in heart rate was 113 ± 14 beats min^{-1} from a resting value of 305 ± 6 beats min^{-1}.

Intact hind limb

In all thirty tests conducted in twelve rabbits stimulation of the nasal mucous membrane caused an increase in MABP from a mean resting value of 84.2 ± 1.3 to 112.4 ± 1.8 mmHg. This response was accompanied by changes in the blood flow to the limb (Fig. 1). Calculation of hind-limb vascular resistance in these tests showed that the vascular response of the hind limb occurred in two well-defined phases: an early phase in which the peak change in vascular resistance occurred within 10 sec of the application of the nasal stimulus and which coincided with the increase in MABP; and a late phase where an increase in vascular resistance oc-curred approximately 50 sec after the stimulus.

Early vascular response. In all thirty tests (twelve rabbits) there was a decrease in hind-limb arterial blood flow following nasal stimulation; the mean reduction in flow was $35.2 \pm 4.1 \%$. In eighteen of these tests (ten animals) however, the response was biphasic, the reduction in flow being

preceded by a transient increase (mean increase $34\cdot0 \pm 6\cdot1\%$, Table 1) concomitant with the period of greatest elevation of the arterial blood pressure. The over-all mean increase in calculated vascular resistance was $91\cdot3 \pm 18\cdot5\%$. In the presence of a constantly changing arterial blood pressure the point of maximum change in hind-limb blood flow did not necessarily coincide with the point of maximum change in calculated vascular resistance. Table 1 includes, therefore, not only an analysis of the maximum changes in blood flow, but also of the blood flow values from which the points of maximum change in calculated vascular resistance were derived.
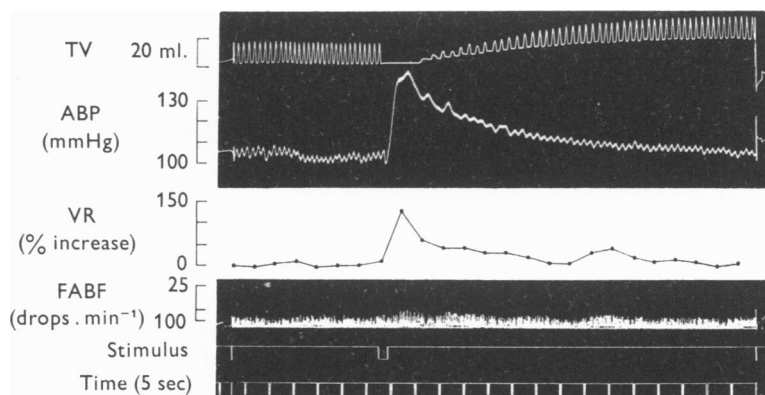

Fig. 1. The effects of stimulation of the nasal mucous membrane with ether on respiration, arterial blood pressure and hind-limb arterial blood flow. The insert shows the percentage change in hind limb calculated vascular resistance. TV: tidal volume, inspiration upwards; ABP: femoral arterial blood pressure; VR: % change in vascular resistance; FABF: femoral arterial blood flow, 10 drops = $1\cdot5$ ml. blood. The stimulus was applied at the point marked.

In four rabbits in which hind-limb perfusion was achieved at constant arterial pressure stimulation of the nasal mucosa still caused significant increases in limb vascular resistance (Table 2).

Late vascular response. In nineteen of the thirty tests (eleven animals) carried out under conditions in which pressure and flow were variable and in which cardiovascular variables were recorded for periods of up to 3 min following stimulation of the nasal mucous membrane a late response was noted consisting of a transient decrease in hind-limb arterial blood flow that was not associated with any significant change in systemic arterial blood pressure. This response attained a peak value between 35 and 70 sec after application of the nasal stimulus (mean $51 \pm 2\cdot8$ sec), and in these nineteen tests there was a mean reduction in blood flow of $17\cdot9 \pm 2\cdot0\%$

TABLE 1. The effects of nasal mucosal stimulation upon arterial blood flow and vascular resistance of the intact, innervated hind limb

	No. of expts.	No. of tests	C	T	Δ	Change (%)	P
1. Early vascular response							
(A) Blood flow (ml. min^{-1})							
(i) Increase	10	18	8.2 ± 0.9	10.8 ± 1.2	$+2.5 \pm 0.5$	$+34.0 \pm 6.1$	< 0.001
(ii) Decrease	12	30	8.8 ± 0.8	5.5 ± 0.6	-3.4 ± 0.5	-35.2 ± 4.1	< 0.001
(iii) At point of max. change in vascular resistance	12	30	9.1 ± 0.7	6.7 ± 0.7	-2.6 ± 0.5	-27.6 ± 4.3	< 0.001
(B) Vascular resistance (mmHg ml.$^{-1}$ min)	12	30	12.7 ± 1.8	21.1 ± 2.5	$+8.7 \pm 1.6$	$+91.3 \pm 18.5$	< 0.001
2. Late vascular response							
(A) Blood flow (ml. min^{-1})	11	19	10.5 ± 0.5	8.8 ± 0.6	-1.8 ± 0.2	-17.9 ± 2.0	< 0.001
(B) Vascular resistance (mmHg ml.$^{-1}$ min)	11	19	8.6 ± 0.6	11.0 ± 0.9	$+2.4 \pm 0.4$	$+25.6 \pm 3.1$	< 0.001

C = control value, T = test value, Δ = absolute difference between control and test values. Change (%) expresses the percentage difference between the control and test values; P = level of significance attached to the absolute difference between control and test values. The values given are the mean values ± s.e. of mean.

TABLE 2. The effects of nasal mucosal stimulation upon hind-limb blood flow and vascular resistance measured at constant arterial blood pressure

	No. of expts.	No. of tests	C	T	Δ	Change (%)	P
(A) Blood flow (ml. min^{-1})							
Method 1	2	13	5·1 ± 0·1	4·0 ± 0·1	−1·1 ± 0·3	−20·5 ± 1·3	< 0·005
Method 2	2	7	5·6 ± 0·2	3·9 ± 0·9	−1·7 ± 0·1	−30·3 ± 1·5	< 0·001
(B) Vascular resistance (mmHg ml.$^{-1}$ min)							
Method 1	2	13	21·7 ± 0·4	27·5 ± 0·7	+ 5·8 ± 0·5	+ 26·6 ± 2·1	< 0·001
Method 2	2	7	15·8 ± 0·7	22·6 ± 1·2	+ 6·8 ± 0·6	+ 43·4 ± 3·3	< 0·001

Method 1: arterial blood pressure maintained at a constant level with a compensating reservoir. Femoral venous blood flow measured.
Method 2: arterial blood pressure maintained at a constant level by manual compression of hind-limb arterial inflow. Femoral arterial blood flow measured.
Notes on column headings as in Table 1.

representing a mean increase in calculated vascular resistance of $25 \cdot 6 \pm$ $3 \cdot 1 \%$ ($P < 0 \cdot 001$). Details of the late vascular response are given in Table 1, and an example is shown in Fig. 1.

In thirteen tests (two animals) in which perfusion was carried out at constant pressure a late vascular response was observed in which the femoral venous blood flow was reduced to $4 \cdot 52 \pm 0 \cdot 13$ ml. min^{-1} from a control value of $5 \cdot 11 \pm 0 \cdot 13$ ml. min^{-1}, representing an increase in vascular resistance of $11 \cdot 6 \pm 1 \cdot 6 \%$.
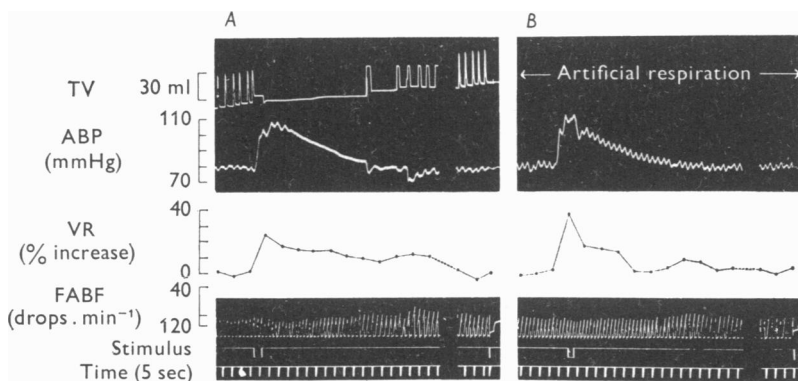

Fig. 2. The effects of stimulation of the nasal mucous membrane with ether on arterial blood pressure (ABP) and hind-limb arterial blood flow (FABF, 10 drops = 1·5 ml. blood) during spontaneous (A) and artificial ventilation (B). The insert shows the percentage change in hind-limb calculated vascular resistance. During the break in each record a period of 20 sec elapsed. The stimulus was applied at the point marked.

Artificial respiration

Since stimulation of the nose in the spontaneously breathing animals invariably caused an immediate apnoea, experiments were conducted using intermittent positive pressure ventilation to investigate how changes in breathing affect the hind-limb vascular responses.

With regard to the early vascular response it was found that in fourteen tests in four animals the changes in hind-limb arterial blood flow evoked by stimulation of the nasal mucous membrane during artificial ventilation followed a similar pattern to that observed in those spontaneously breathing animals in which concomitant apnoea took place (Fig. $2A$, B). A decrease in blood flow was observed in all fourteen tests from a resting value of $7 \cdot 9 \pm 0 \cdot 8$ ml. min^{-1} to a value of $6 \cdot 6 \pm 0 \cdot 7$ ml. min^{-1}. This represents a mean reduction in flow of $15 \cdot 6 \pm 3 \cdot 1 \%$ ($P < 0 \cdot 005$). The increase in the calculated vascular resistance was $4 \cdot 0 \pm 0 \cdot 6$ mmHg ml.$^{-1}$ min from a control value of $12 \cdot 8 \pm 2 \cdot 4$ mmHg ml.$^{-1}$ min, representing an increase of $38 \cdot 3 \pm 7 \cdot 9 \%$ ($P < 0 \cdot 001$).

The maximum changes in hind-limb vascular resistance following nasal stimulation with and without artificial ventilation was $38 \cdot 3 \pm 7 \cdot 9 \%$ ($n = 14$) and $91 \cdot 3 \pm 18 \cdot 5 \%$ ($n = 30$) respectively. This result suggests that the apnoea occurring during nasal stimulation may augment the effects of the stimulus on the hind-limb vascular resistance ($P < 0 \cdot 05$).

A delayed vascular response similar in time course to that observed in animals undergoing nasal stimulation with concomitant apnoea was also seen in animals stimulated during positive pressure ventilation. In eleven tests (four animals) the late response was maximal at $53 \pm 5 \cdot 3$ sec after stimulation of the nose (Fig. 2*B*). The mean decrease in blood flow was $14 \cdot 5 \pm 1 \cdot 4 \%$ which represents an increase in vascular resistance of $16 \cdot 2 \pm 2 \cdot 0 \%$ from a control value of $9 \cdot 4 \pm 1 \cdot 0$ mmHg ml.$^{-1}$ min.

Skinned hind limb

Early vascular response. The pattern of the blood flow changes was similar to that obtained in the intact limb; there was a reduction in arterial blood flow observed in all nine tests (five animals) following stimulation of the nose, corresponding to an increase in the calculated vascular resistance of $32 \cdot 4 \pm 4 \cdot 6 \%$ ($P < 0 \cdot 005$; Table 3).

Late vascular response. In four tests (two animals) a late reduction in blood flow and increase in vascular resistance was observed (Table 3). The peak of the response occurred at $45 \pm 2 \cdot 0$ sec after stimulation compared with a time of $51 \pm 2 \cdot 8$ sec in the intact hind limb. Table 3 shows the results obtained in twenty-three tests in three animals with skinned hind limbs in which venous blood flow was recorded. The early and late increases in vascular resistance obtained during stimulation of the nose in these tests were very similar to those obtained in the tests in which arterial blood flow was measured.

Afferent and efferent pathways of the response to nasal stimulation

In nine tests (seven animals) the respiratory and vasomotor responses to ether stimulation of the nose were abolished by local anaesthetic applied to the nasal passages. In two animals the response returned after sufficient time had elapsed for the effects of the local anaesthetic to wear off.

The efferent pathway mediating the vascular responses to nasal stimulation was studied in three animals in which the femoral and sciatic nerves to one hind limb had been divided. In seven tests the vascular responses in the denervated limb were compared with the responses in the opposite intact limb.

Hind-limb denervation abolished the early increase in vascular resistance to stimulation of the nasal mucosa; arterial blood flow increased following such stimulation and occurred during the phase of elevated

TABLE 3. The effects of nasal mucosal stimulation upon blood flow measured in the femoral artery (arterial) or femoral vein (venous) and upon vascular resistance of the skinned, innervated hind limb

|	No. of expts.	No. of tests	C	T	Δ	Change (%)	P
1. Early vascular response							
(A) Blood flow (ml. min⁻¹)							
Arterial	2	9	6·0±0·8	5·4±0·9	−0·65±0·3	−12·0±4·9	> 0·05
Venous	3	27	5·8±0·1	6·4±0·2	+0·6±0·1	+10·9±2·0	< 0·001
(B) Vascular resistance (mmHg ml.⁻¹ min)							
Arterial	2	9	14·7±1·9	19·5±2·6	+4·8±0·9	+32·4±4·6	< 0·005
Venous	3	27	15·7±0·3	21·0±0·5	+5·3±0·4	+33·8±2·4	< 0·001
2. Late vascular response							
(A) Blood flow (ml. min⁻¹)							
Arterial	2	4	7·8±1·3	6·0±1·0	−1·7±0·3	−15·5±1·5	—
Venous	3	23	5·8±0·1	5·4±0·1	−0·4±0·06	−7·1±1·0	< 0·001
(B) Vascular resistance (mmHg ml.⁻¹ min)							
Arterial	2	4	12·8±2·8	14·5±3·0	+1·7±0·4	+14·3±3·1	—
Venous	3	23	15·6±0·3	17·3±0·5	+1·75±0·3	+11·3±2·0	< 0·001

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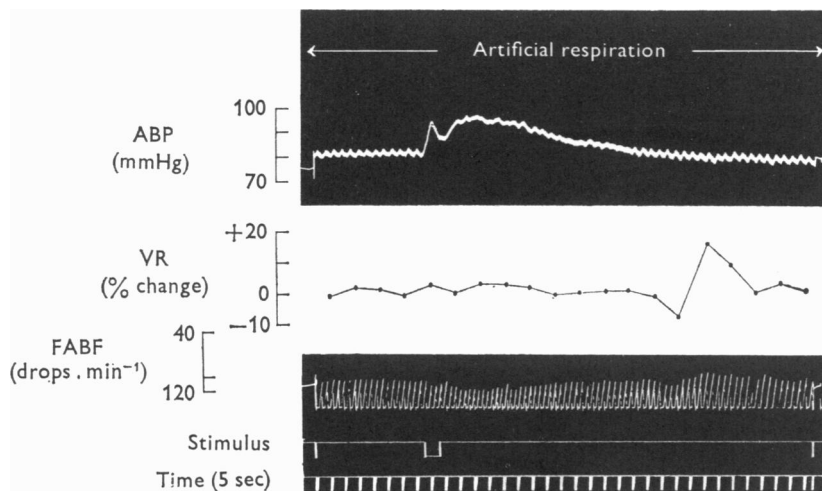
Fig. 3. The effects of stimulation of the nasal mucous membrane with ether on arterial blood pressure (ABP) and arterial blood flow (FABF) in the denervated hind limb during artificial ventilation. The insert shows the percentage change in hind-limb calculated vascular resistance (VR). The stimulus was applied at the point marked.
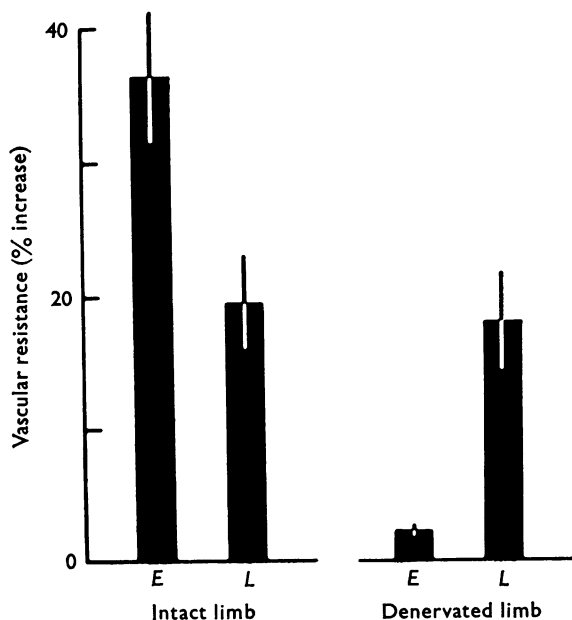

Fig. 4. Comparison of the immediate and delayed effects evoked by stimulation of the nasal mucous membrane with ether on the vascular resistance of intact and denervated hind limbs of the same animals. Vertical lines show s.e. of mean. *E*, early vascular response; *L*, late vascular response.

MABP (Fig. 3). By contrast, however, the early increase in vascular resistance still occurred in the innervated hind limb. The mean increase in the seven tests was $36\cdot4 \pm 4\cdot9\%$ ($P < 0\cdot001$).

In both intact and denervated limbs a late vascular response was observed. In the intact limb the peak of this late response occurred $46 \pm 5\cdot1$ sec after stimulation of the nose and in the denervated limb the peak response occurred at $48 \pm 4\cdot7$ sec. At this time after stimulation the MABP had in most tests returned to its resting value and the response was manifest by a reduction in hind-limb blood flow in the presence of a constant perfusion pressure. In the intact limbs the mean reduction in flow was $14\cdot7 \pm 2\cdot6\%$ ($P < 0\cdot001$), corresponding to an increase in vascular resistance of $19\cdot4 \pm 3\cdot5\%$ ($P < 0\cdot0025$). In the denervated limbs the mean reduction in flow was $14\cdot9 \pm 1\cdot7\%$ ($P < 0\cdot001$) representing an increase in vascular resistance of $17\cdot7 \pm 3\cdot7\%$ ($P < 0\cdot001$; Fig. 4).
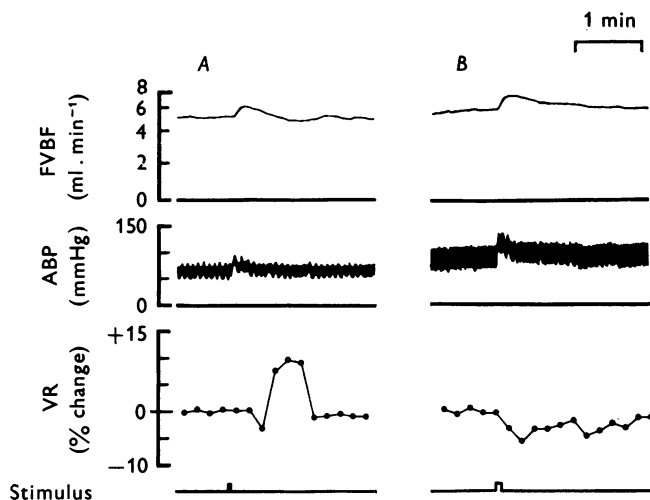

Fig. 5. The effects of stimulation of the nasal mucous membrane with ether on arterial blood pressure (ABP) and femoral venous blood flow (FVBF) in a sympathetically denervated hind limb before (A) and after (B) occlusion of the venous drainage from the right and left adrenal glands. Trace B was obtained approximately 45 min after trace A. The insert shows the percentage change in hind-limb calculated vascular resistance. The stimulus was applied at the point marked.

Similar results occurred in three animals in which the sympathetic supply to the hind limb was cut at L4; the early response was abolished whereas the late response was unaffected (Fig. 5).

Adrenal venous occlusion. In three tests in three animals with innervated hind limbs and in three tests in three animals with denervated limbs the late increase in vascular resistance invariably obtained after nasal stimula-

tion was abolished following bilateral occlusion of the adrenal veins (Fig. 5*B*). Adrenal venous occlusion resulted in no measurable change in either the magnitude or duration of the early vascular response.

<div align="center">DISCUSSION</div>

Stimulation of the nasal mucous membrane in the rabbit with ether vapour leads to pronounced cardiovascular and respiratory effects similar to those which have been described in other mammalian species (see Angell-James & Daly, 1969, for references). In the present experiments such stimulation produced an apnoea in the expiratory position, brady-cardia and a rise in arterial blood pressure. Stimulation of the nose resulted also in an increase in hind-limb vascular resistance which was shown to be due to vasoconstriction since this increase was still observed when changes in arterial blood pressure were prevented (Table 2).

The vasoconstrictor response in both the intact and in the skinned hind limb occurred in two distinct phases following stimulation: an early phase manifest within 10 sec of the application of the stimulus and coinciding with the period of maximally increased arterial blood pressure; and a late phase, less pronounced and occurring approximately 50 sec after stimula-tion of the nose when the early phase had invariably passed and the blood pressure had returned to resting levels.

Reflex nature of the hind-limb vascular response. Absorption of inhaled gases is known to occur readily in the nose (Malcolmson, 1959; Brain, 1970) and it could be contended therefore that the ether used to stimulate the nose is absorbed, and subsequently evokes the characteristic responses by its action either on the central nervous system or on the end organs. It would appear, however, that the responses to nasal stimulation are almost certainly too rapid in onset to be accounted for entirely in this way (e.g. Figs. 1 and 2) and that the abolition of the responses by local anaes-thesia of the nasal mucous membrane, while not completely excluding such a mechanism, makes it exceedingly improbable. Furthermore quali-tatively identical respiratory and cardiovascular responses can be evoked in the anaesthetized rabbit by drawing water or saline across the nasal mucous membrane (Allison & Powis, 1971; Allison, 1974).

It was established that both the early and late hind-limb vascular responses were still evoked during artificial ventilation (Figs. 2 and 4) and could not therefore be attributed entirely to changes in the blood gas tensions nor to changes in pulmonary vagal reflex activity consequent upon the arrest of breathing in the expiratory position (Daly, Hazzledine & Ungar, 1967; Daly & Robinson, 1968; Ott, Lorenz & Shepherd, 1972; Angell-James & Daly, 1972).

The early vasoconstrictor response of the hind limb occurred during a period when the systemic arterial blood pressure was elevated and the late constrictor response occurred at a time when the blood pressure was either still slightly elevated or had returned to its resting level. In neither case therefore could the vasoconstrictor responses be accounted for as part of an arterial baroreceptor reflex since under these circumstances any baroreceptor activity would be opposing the observed constrictor response rather than contributing to it.

These observations together with the finding that both early and late vascular responses were abolished by local anaesthesia of the nasal mucous membrane prior to nasal stimulation suggest that hind-limb vasomotor changes are due to activation of primary reflex pathways between the nose and nervous and/or humoral effector mechanisms.

Efferent pathways of the hind-limb vascular response. The early phase of the hind-limb vasoconstrictor response was invariably abolished either by hind limb denervation or by lumbar sympathectomy, suggesting that it is mediated by efferent fibres of the sympathetic nervous system. The late vasoconstriction cannot however be mediated by the sympathetic nerves to the limb since this phase persists undiminished after denervation of the organ (Fig. 5*A*). The observation that the late vasoconstrictor response did not occur following adrenal venous occlusion suggests that the secondary effects are due mainly to circulating adrenal gland hormones.

Previous work in the rabbit has shown that the vascular effects produced in the hind limb by catecholamines and by the sympathetic nerves are additive (Powis, 1974) but it would appear that in the present experiments the time course over which each sympathetic component exerts its peripheral vascular action following nasal mucosal stimulation is such that mutual reinforcement of the effects does not occur (see above). It might be argued that the delay between the early and late phases of the measured vasoconstrictor response was unnaturally long due to the inclusion of the extracorporeal blood flow measuring device in the hind-limb arterial inflow. However a delay of similar duration was observed also in those experiments where hind-limb blood flow was estimated in the femoral vein and the arterial inflow to the limb was not interrupted.

Adrenal gland hormones. An increase in adrenal medullary activity is known to occur in the rabbit during nasal mucosal stimulation with ether and the major component of the induced secretion is adrenaline (Allison & Powis, 1971), an amine which, at concentrations sufficient to exert vasomotor effects, produces only vasoconstriction in the hind-limb vasculature of the rabbit (Powis, 1974). The latter author showed additionally that in the quantities liberated from the adrenal glands during nasal mucosal stimulation, adrenaline could produce a vasoconstriction in the hind limb

similar in magnitude to that constituting the late vascular response reported here. It has been shown that the predicted delay between stimulation of the nasal mucous membrane in the anaesthetized rabbit and any subsequent effect on cardiovascular parameters due to catecholamines released by such stimulation is within the range 37–75 sec (Allison, 1974), an estimate which correlates well with the timing of the late onset constrictor response in the present study. A delayed vasoconstrictor response, persisting after denervation of the organ, has been reported in the renal vascular bed following stimulation of the nose in rabbits (White & Franklin, 1970) and in the light of the present evidence it seems likely that this can also be ascribed to the increased secretion of catecholamines by the adrenal medulla. It is of interest to note in this context that there may be some species variation in the nature of the peripheral vascular response to nasal stimulation: in the dog where adrenaline at low concentrations causes vasodilatation, Angell-James & Daly (1972) reported a late vasodilatation in the femoral vascular bed of dogs following nasal mucosal stimulation which they considered could be attributable to catecholamines.

The delayed constrictor response observed in the hind limb and attributed to catecholamines was less pronounced than the early response in every test; the contribution of circulating catecholamines to the over-all changes recorded in other cardiovascular variables following nasal stimulation is probably also small. The pressor response to stimulation of the nose was unaffected by adrenal venous occlusion, as was the degree of bradycardia. The cardiovascular effects attributable to the increased adrenal medullary activity evoked by nasal stimulation seem negligible therefore, in comparison with those produced by the neurogenic components of the response. This preponderance of sympathetic neurogenic effects on blood vessels over sympathetic humoral effects has also been noted, albeit in different reflex situations, in the cat (Celander, 1954).

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