J. Physiol. (1972), **220**, *pp.* 673–696
With 9 text-figures
Printed in Great Britain

REFLEX RESPIRATORY AND CARDIOVASCULAR EFFECTS OF STIMULATION OF RECEPTORS IN THE NOSE OF THE DOG

By JENNIFER E. ANGELL JAMES and M. de BURGH DALY
From the Department of Physiology, St Bartholomew's Hospital Medical College, London, EC1M 6BQ

(*Received 6 August 1971*)

SUMMARY

1. In forty-one out of forty-seven dogs under chloralose–urethane or Nembutal anaesthesia, mechanical stimulation of the nasal mucous membrane caused a reduction or inhibition of respiration, bradycardia, variable changes of arterial blood pressure, and a small rise in venous pressure.

2. Simultaneous measurements of arterial and venous pressures, and also of blood flow in various arteries by means of an electromagnetic flowmeter indicate that the calculated vascular resistance increases in the intact limb, muscle, and skin, and the vascular beds of the vertebral, superior mesenteric, renal and splenic arteries. No changes in vascular resistance occur in the common carotid circulation.

3. Evidence is presented that the increase in vascular resistance is due to vasoconstriction, and occurs in the absence of changes in pulmonary ventilation.

4. Stimulation of the nasal mucous membrane causes a reduction in volume of the spleen.

5. The respiratory and cardiovascular responses are reflex in nature, being abolished by the application of a local anaesthetic to the nose or by combined division of the maxillary and ethmoidal branches of the trigeminal nerves. The cardiac response is mediated largely by the vagus nerves, and the vascular responses by sympathetic adrenergic fibres.

6. Cessation of the stimulus to the nose not infrequently results in the following temporary after-effects: hyperventilation, tachycardia, hypertension, and vasodilatation in the intact limb and in muscle.

INTRODUCTION

It has been shown previously in a number of animal species that mechanical, electrical and chemical stimulation of receptors in the nose usually cause a reflex inhibition of respiration in the expiratory position and bradycardia (Kratschmer, 1870; Brodie & Russell, 1900; Dixon & Brodie, 1903; Allen, 1928; Angell James & Daly, 1969). Similar observations have been made on man (Francois-Franck, 1889) although mechanical irritation of the nasal mucous membrane may cause tachycardia rather than bradycardia (Corbett, Kerr & Prys-Roberts, 1969).

So far as we are aware, there is no information as to whether these respiratory and cardiac responses are accompanied by any changes in systemic vascular resistance. In this paper we describe the results of experiments which demonstrate that in the dog stimulation of the nasal mucous membrane causes reflex vasoconstriction in some vascular territories, but not in others. Some of our results have been reported briefly elsewhere (Angell James & Daly, 1969).

METHODS

Dogs of either sex varying in weight from 10·4 to 30·5 kg were anaesthetized with morphine hydrochloride (1 mg/kg s.c.) followed 30 min later by an I.V. injection of a mixture of α-chloralose (Kuhlmann, Paris; 0·05 g/kg) and urethane (British Drug Houses, Ltd.; 0·5 g/kg) dissolved to make a solution of 10 g α-chloralose and 100 g urethane in 100 ml. of a solution containing 85 parts sodium chloride sodium (0·9 g/100 ml.) and 15 parts polyethylene glycol ('Carbowax', Union Carbide Ltd.). In a few experiments pentobarbitone sodium (Macarthy) was used in a dose of 35–40 mg/kg I.V.

Stimulation of receptors in the nose. Liquids or gases were drawn over the nasal mucous membrane as described by Angell James & Daly (1969). A cuffed tube was inserted into the trachea, above the tracheotomy tube, towards the nose so that the lip lay in the nasopharynx at the level of the caudal edge of the hard palate. The cuff was inflated immediately before recording began. A mask was put over the muzzle and sealed. The cuffed tube was attached to a suction line and liquids (tap water or sodium chloride solution, 0·9 g/100 ml.) were drawn through the nose at a constant rate which varied from 0·2 to 1·0 l./min in different experiments.

Further localization of the stimulus to the nasal mucous membrane itself was achieved in experiments in which the mask over the muzzle was replaced by a tube inserted about 1 cm into each nostril to avoid stimulating receptors in the external nose. The cuffed tube in the nasopharynx was replaced by two smaller cuffed tubes, one inserted through each choana and nasopharyngeal meatus to lie with its tip in the common nasal meatus. In this way the stimulus was localized to the nasal mucous membrane.

The inflow and, in some experiments, the outflow temperatures were measured by mercury thermometers. Unless otherwise stated the inflow temperature was that of room air (20–24° C) when the outflow temperature was found to be 1–2° C higher. When tests were made at lower inflow temperatures (down to 4° C), the outflow

temperature was up to 3° C higher. On the other hand, at an inflow temperature of 37° C, the outflow temperature was the same.

The reflex responses from the nose were prone to diminish and disappear spontaneously unless certain precautions were taken. It was necessary to ensure that light anaesthesia was maintained (Allen, 1936) and that the venous drainage from the nose was not restricted such as by having the head in a dependent position. With the animal usually in the supine position on the operating table venous drainage was facilitated by elevating the head on a platform 25° to the horizontal. Again in experiments in which the animal was in the prone position for exposure of the trigeminal nerves, the head was held above the level of the trunk. Gases in excessive concentrations also diminished or abolished the responses, as found by Dixon & Brodie (1903).

Exposure of the branches of the trigeminal nerves. In some experiments the maxillary division of the trigeminal nerve and the ethmoidal nerve were exposed on each side using a trans-orbital approach. The animal was held in the prone position and dissection of the nerves was facilitated by removing the eyeball and widely opening the mouth. The latter procedure depressed the coronoid process of the mandible and the muscles attached to it, which gave better access to the nerves.

Respiration. Tidal volume was measured by a balanced spirometer connected to a closed-circuit respiratory system (Bacon, Daly & Scott, 1962). The counter-weight for the spirometer formed the core of a linear displacement transducer (S. E. Laboratories Ltd., Feltham, Middlesex) which was connected to a carrier amplifier (S. E. Laboratories, Ltd.). The animals breathed 100% oxygen throughout each experiment.

Measurement of pressures. Arterial blood pressure was measured from a brachial or femoral artery by way of a flexible nylon catheter (Portex, Portland Plastics Ltd.; length 20 cm, bore 1·5 mm). Mean right atrial or mean inferior vena caval pressure was measured via a flexible nylon catheter (length 20 cm, bore 1·5 mm) inserted through a branch of the right external jugular vein or the left femoral vein respectively. Each pressure was measured by means of a Statham strain-gauge (model P23Gb), the output of which was connected to a carrier amplifier (S.E. Laboratories Ltd). The frequency response of the arterial catheter-manometer system was determined using the method of Frank (1903). The undamped natural frequency response was greater than 130 c/s, the degree of damping being 0·2. This gave an estimated distortion of less than 5% up to about 40 c/s. Mean arterial pressure was obtained electrically by passing the output of the amplifier through a simple R-C network with a time constant of 1 or 2 sec, and was recorded separately. The manometers were calibrated before and after each experiment using a mercury manometer. Zero reference pressures were obtained post mortem and taken as those recorded when the tips of the catheters were exposed to air.

Heart rate. This was recorded continuously using a pulse frequency meter (J. F. Tonnies, Frieberg im Breisgau, Western Germany) which was triggered by the output signal of the Statham strain-gauge measuring arterial blood pressure. Measurements of heart rate were made over a 10 or 20 sec period from the blood pressure record.

Regional blood flow. Measurements of mean blood flow were made in the following arteries: common carotid, vertebral, femoral, superior mesenteric, renal and splenic. For this purpose a two-channel electromagnetic flowmeter (Nycotron, Oslo, Norway) was used with non-cannulating transducers. Zero reference flow was obtained at intervals during each experiment by temporary occlusion of the vessel distal to the transducer. In some experiments, phasic blood flow was measured as well as the mean flow. The electrical response of the instrument and recorder was flat $\pm 5\%$ up to 10 c/s, and it is reasonable to assume it is the same for the hydraulic frequency response (Gessner & Bergel, 1964).

At the end of each experiment the probe was calibrated *in situ*. For this purpose the vessel was cannulated distally and the animal's own blood was run into a measuring cylinder at different rates which were timed with a stopwatch. In all cases there was a linear relation between blood flow and galvanometer deflexion, the line passing through the point of origin.

Muscle blood flow was measured in a femoral artery of the skinned limb, the paw being severed at the level of the ankle joint.

Blood flow to a hind-limb paw, which comprises predominantly skin, was measured in an anterior tibial artery and plantar branch of the saphenous artery by means of a photo-electric drop counter (Lindgren, 1958) and drop timer (Gaddum & Kwiatkowski, 1938), the latter operating a linear displacement transducer (S. E. Laboratories Ltd.). The counter was calibrated in ml./min at the end of each experiment by collecting timed samples of blood.

Spleen volume. The spleen was exteriorized through a mid-line abdominal incision and placed in a plethysmograph as described by Daly & Scott (1961). To immobilize the spleen sufficiently some of its vascular connexions with the stomach were severed. Changes in volume were measured with a volume recorder operating a linear displacement transducer.

All parameters were recorded on a direct-writing ultra-violet light recorder (Type S.E. 2100, S.E. Laboratories Ltd.).

Measurement of vascular resistance

In most experiments regional vascular resistance was calculated as

$$\frac{\text{(mean arterial blood pressure minus venous pressure) mm Hg}}{\text{blood flow (ml./min)}}$$

and expressed in convenient units (peripheral resistance units) representing the pressure necessary to force blood at 1 ml./min through the vascular bed under test. Changes in venous pressure on stimulation of the nasal membrane were relatively small and in a few experiments in which it was not measured, the regional vascular resistance was expressed as arterial blood pressure/blood flow.

Perfusion at constant blood flow. In two experiments the upper or lower limb was perfused at constant flow by means of roller pump (type MHRE, Watson Marlow Ltd.). To exclude collateral blood flow a tape was temporarily tightened round the upper part of the lower limb during each test. In the case of the upper limb, vascular isolation was achieved by ligating all the muscle masses and dividing the humerus; its innervation was preserved. Perfusion was carried out through the brachial artery.

Pressure–flow curves. Pressure–flow curves of the femoral vascular bed were determined before, during and after stimulation of the nasal mucous membrane using a modification of the method described by Cassin, Dawes, Mott, Ross & Strang (1964). The apparatus used is shown in Fig. 1. Blood from the left femoral artery perfused the vascular bed of the right femoral artery via the condom rubber sleeve (*a*). The sleeve had a maximum capacity of 25 ml. and was held in an unstretched state, distension by the pressure within it being prevented by the perforated Perspex tube (*b*). The rubber sleeve and Perspex tube were held in the Perspex chamber (*c*) containing water which was maintained at a constant temperature of 38° C. The perfusion pressure was measured by a Statham strain-gauge manometer (*d*) and the blood flow by an electromagnetic flow meter (*e*). The space between the Perspex tube (*b*) and chamber (*c*) was connected to a 5-l. air-reservoir pumped up initially to a pressure of 120 mm Hg. An air-leak by-pass (*f*) was provided for subsequently controlling the rate of decline of the air-reservoir pressure. When perfusion was from the

left femoral artery, this air-leak by-pass and the tube connecting the air-reservoir with the Perspex chamber (*c*) were occluded at point (*g*).

The pressure flow relationship was determined by simultaneously occluding the femoral arterial tubing at (*h*) and removing the clamp at (*g*). The outside of the rubber sleeve was now subjected to the air-reservoir pressure which gradually declined to 50 mm Hg over a period of 12–25 sec by the escape of air through tube (*f*). When the pressure had decayed, normal blood flow was restored by reversing the procedure, namely by removing the clamp at (*h*) and re-applying the clamp at (*g*). Curves were plotted from instantaneous measurements of arterial pressure and blood flow. To minimize collateral blood flow to the limb in these experiments, a tape was temporarily drawn tightly round the upper part of the limb, the femoral artery and vein lying on the outside of the tape.

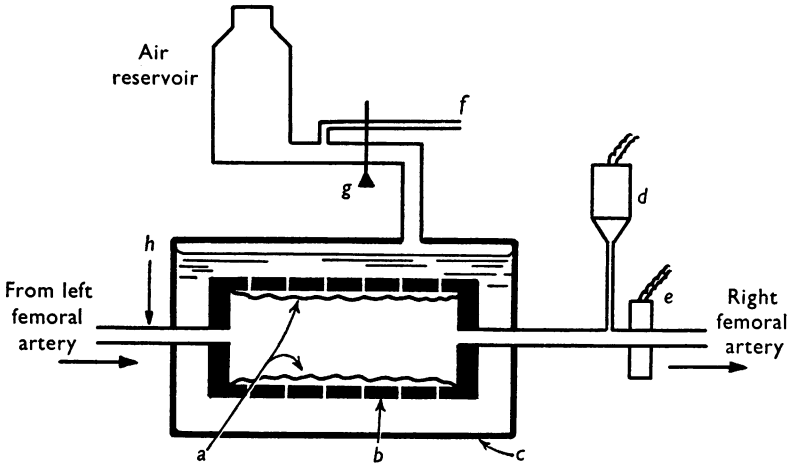

Fig. 1. Diagram showing method of determining the pressure–flow curve of the femoral vascular bed. *a*, condom rubber sleeve; *b*, perforated Perspex tube; *c*, Perspex chamber; *d*, Statham strain-gauge manometer; *e*, blood flow probe; *f*, air-leak by-pass; *g*, position of clamp on two tubes; *h*, position of clamp on femoral arterial tubing. The horizontal arrows indicate the direction of blood flow. For details see text.

Blood gas analysis

Samples of arterial blood were withdrawn anaerobically and P_{O_2} and P_{CO_2} were determined using an oxygen electrode (Electronic Instruments Ltd) and a Severinghaus CO_2 electrode in conjunction with a pH measuring unit (Model C33B) and a Vibron electrometer (Model 33B, Electronic Instruments Ltd). The pH of the blood was measured using a capillary glass electrode (Type SHH33, Electronic Instruments Ltd) (Daly & Ungar, 1966).

While the surgical procedures were being carried out, an infusion of a mixture of four parts of Dextran ('Macrodex 6%' in sodium chloride solution (0·9%, w/v), Pharmacia, Uppsala) and one part of sodium bicarbonate solution (20 mM) was administered intravenously at a rate of 2–3 ml./kg.hr to maintain a normal acid-base balance.

Drugs

When necessary animals were given heparin (Pularin, Evans Medical Ltd, 1000 i.u./kg) to render the blood incoagulable. Other drugs used were: atropine sulphate (British Drug Houses Ltd.), hexamethonium bromide (May & Baker Ltd.), bretylium tosylate ('Darenthin', Burroughs Wellcome Ltd), and propranolol hydrochloride ('Inderal', Imperial Chemical Industries Ltd).

RESULTS

The effects of stimulation of the nasal mucous membrane

A total of forty-seven experiments were performed, but in six of these stimulation of the nasal mucous membrane had no effect on either respiration or the cardiovascular system, so that the results described in this paper are based on forty-one experiments in which reflex responses were observed. The results of not more than four control stimulations of the nose per experiment were included for analysis to prevent those of a few experiments producing undue bias in the averaged results. For the same reason the number of stimulations per experiment following an intervention was limited to four.

Although in the majority of experiments the method used for stimulating the nose by water or saline consisted of using the mask over the muzzle and inflated cuff in the nasopharynx, it was found that similar effects on respiration, heart and vascular resistance were obtained in experiments using the alternative method of localizing the stimulus to the nasal mucous membrane itself (see Methods). However, the mere presence of water or saline in the nose was not sufficient to elicit any responses; the liquid had to be in motion.

Respiration. Stimulation of the nasal mucous membrane by water or saline at room temperature caused a reduction of breathing or apnoea in the expiratory position. The duration of apnoea was variable and lasted from 10 to 40 sec before the 'break-through' occurred. The 'break-through' consisted of irregular breathing accompanied occasionally by convulsive movements of the animal. These convulsions often dislodged the flow probes and so the stimulus was usually applied only for a length of time sufficient to obtain sizable responses and this was determined at the beginning of each experiment. Thus we did not obtain in all experiments a value for the maximum apnoeic period. Typical responses are shown in Figs. 2, 6, 7, 8*A* and 9.

Heart rate. In 114 tests in thirty experiments, stimulation of the nasal mucous membrane caused either a reduction in rate (ninety-nine tests in thirty experiments) or no change (fifteen tests in six experiments). In six of the thirty experiments, therefore, there was a mixed response.

Considering all tests together, there was a mean reduction in heart rate

of $31{\cdot}3 \pm 2{\cdot}1$ beats/min (mean $\pm$ S.E. of mean, range 0–105), the initial control rate being $104{\cdot}4 \pm 13{\cdot}2$ beats/min (range 60–170). This represents a reduction in rate of $29{\cdot}9 \pm 1{\cdot}7\%$ (range 0–78). Typical responses are shown in Figs. 2, 6, 7*A*, 8*A* and 9.

Arterial blood pressure. The mean arterial blood pressure changes were variable and depended to a large extent on the alterations in heart rate. In the majority of experiments there was an initial fall in pressure which was accentuated when the heart rate fell precipitously. This was followed by a gradual recovery of the pressure, occasionally to a level in excess of the control value. At a time when the vasomotor response in the vascular territory under test was maximal, there was a mean fall in mean arterial blood pressure of $5{\cdot}7 \pm 1{\cdot}6$ mm Hg (range -65 to $+36$; 114 tests in thirty experiments; $P < 0{\cdot}001$), the initial control level being $128{\cdot}3 \pm 1{\cdot}1$ (range 98–164). This represents a reduction in pressure of $4{\cdot}2 \pm 1{\cdot}2\%$ (range -41 to $+20$).

Venous pressure. In eighteen experiments it was found that the mean right atrial pressure or inferior vena caval pressure rose up to 5 mm Hg in response to stimulation of the nasal mucous membrane. The rise in pressure was more evident in those experiments in which there was a profound slowing of the heart.

Changes in regional vascular resistance

Intact limb. In seventy-four tests in twenty-five experiments in which blood flow in a femoral artery was measured, there was a reduction in mean blood flow of $46{\cdot}2 \pm 1{\cdot}9\%$ in response to stimulation of the nasal mucous membrane (Table 1). The mean calculated vascular resistance increased by $97{\cdot}9 \pm 8{\cdot}3\%$ ($P < 0{\cdot}001$, Table 1). Typical responses are shown in Figs. 2, 6, 8*A* and 9, and the time course of the change in vascular resistance is shown for one experiment in Fig. 2.

These changes in limb vascular resistance occurred when the changes in mean arterial pressure (Table 1) and in venous pressure were minimal, and are unlikely therefore to be due to passive effects of alterations in hydrostatic pressure within the vessels. Support for this view was obtained in three different types of experiment:

1. In some tests of stimulation of the nasal mucous membrane, the arterial blood pressure, after an initial rise or fall, returned to its original level before the stimulus was withdrawn. Calculations of the limb vascular resistance were therefore made from the values for blood flow at levels of arterial pressure which were the same before, during and after stimulation of the nose. The results are summarized in Table 2 showing that the limb vascular resistance increased by $122{\cdot}2 \pm 20{\cdot}2\%$ in twenty-four tests in nine experiments.

TABLE 1. The effects of stimulation of the nasal mucous membrane on the calculated resistance of various vascular territories (arterial pressure minus venous pressure/blood flow)

Vascular bed	No. of expts.	No. of tests	Mean arterial B.P. Control (mm Hg)	Mean arterial B.P. Change (mm Hg)	Mean arterial B.P. Change (%)	Mean blood flow Control (ml./min)	Mean blood flow Change (ml./min)	Mean blood flow Change (%)	Vascular resistance Control (mm Hg/ml./min)	Vascular resistance Change (mm Hg/ml./min)	Vascular resistance Change (%)
Intact lower limb	25	74	130·9±1·5 (100–164)	−5·0±2·1 (−65 to +36)	−3·7±1·6 (−41 to +31)	68·7±4·7 (15–210)	−32·0±2·4 (−7 to −75)	−46·2±1·9 (−13 to −80)	2·67±0·20 (0·57–9·70)	2·48±0·32 (0·22–10·9)	97·9±8·3 (16–362)
Lower limb (muscle)	3	12	138·2±5·0 (115–154)	−3·2±4·1 (−28 to +12)	−1·9±2·9 (−19 to +10)	43·3±4·4 (18–67)	−22·8±2·4 (−12 to −37)	−58·4±6·9 (−25 to −86)	3·72±0·47 (1·72–7·10)	9·40±2·40 (0·76–23·6)	222·3±52·6 (41–542)
Lower limb paw (skin)	3	12	130·3±2·0 (120–145)	−0·7±3·66 (−16 to +26)	−0·3±2·8 (−12 to +20)	8·2±0·7 (7·0–9·5)	−1·7±0·27 (0 to −3·0)	−23·6±3·9 (0 to −38)	17·5±1·3 (9·6–26·0)	5·9±0·91 (1·5–12·3)	32·5±3·6 (13–55)
Common carotid	8	28	128·6±1·6 (112–145)	−8·7±4·0 (−50 to +26)	−6·5±3·0 (−36 to +20)	120·2±5·0 (65–180)	−4·7±8·0 (−80 to +90)	−2·7±6·1 (−55 to +60)	1·12±0·04 (0·74–1·75)	0·05±0·06 (−0·31 to +0·43)	1·8±4·1 (−33 to +45)
Vertebral	5	17	126·5±1·2 (118–134)	−12·2±3·9 (−32 to +16)	−9·4±3·1 (−24 to +13)	71·5±4·4 (45–120)	−20·8±3·7 (0 to −50)	−29·6±4·4 (0 to −59)	1·87±0·11 (1·12–2·85)	0·76±0·20 (−0·18 to +2·48)	38·3±9·5 (−9 to +105
Mesenteric	3	7	120·1±3·7 (112–140)	9·3±2·7 (−6 to +15)	7·7±2·3 (−5 to +13)	133·6±24·8 (90–280)	−31·6±7·6 (−6 to −65)	−23·4±4·8 (−7 to −43)	0·98±0·10 (0·50–1·36)	0·45±0·12 (0·20–1·11)	47·6±12·6 (19–116)
Splenic	2	5	122·0±2·0 (120–130)	−8·0±4·9 (−22 to 0)	−6·4±4·0 (−18 to 0)	78·0±7·3 (60–100)	−26·0±8·8 (−10 to −50)	−29·6±7·3 (−17 to −45)	1·64±0·14 (1·30–2·00)	0·46±0 08 (0·24–0·73)	30·8±8·2 (13–56)
Renal		15	128·7±2·0 (112–142)	8·5±6·0 (−24 to +42)	6·9±4·9 (−19 to +33)	288·3±10·2 (225–340)	−33·7±7·4 (0 to −80)	−12·8±2·9 (0 to −31)	0·46±0·02 (0·35–0·59)	0·10±0·01 (0·03–0·21)	22·6±2·8 (7–40)

The open values are the means ±S.E. of mean, those in parentheses the range.

2. In one experiment the lower limb was perfused at constant blood flow by means of a pump, collateral blood flow being excluded by a tape drawn tightly round the upper part of the limb. Stimulation of the nasal mucous membrane caused a rise in arterial perfusion pressure. A similar response was obtained in another experiment involving perfusion of the upper limb separated from the trunk of the animal with the exception of its nerve supply.

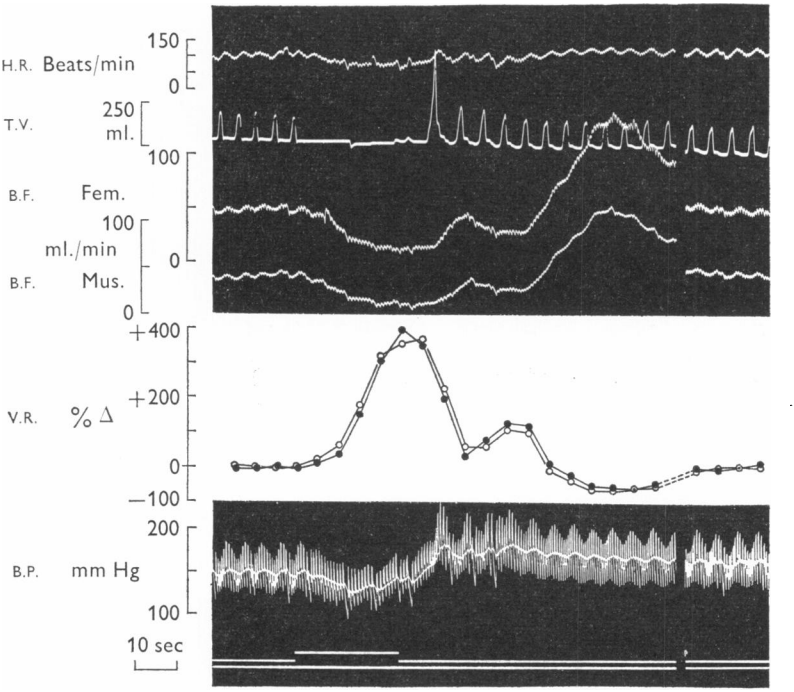

Fig. 2. Effects of stimulation of the nasal mucous membrane by water at 4° C on blood flow and calculated vascular resistance in the normal and skinned lower limbs. Dog, male, 14·3 kg. Morphine-chloralose-urethane anaesthesia. The insert shows the percentage change calculated vascular resistance in the normal left lower limb (*filled circles*, ●) and in the right skinned limb (muscle) (*open circles*, ○). During the break in the records, a period of 2 min elapsed. Records from above downwards: heart rate (H.R.), tidal volume (T.V.), left femoral blood flow in normal limb (B.F. Fem.), right femoral blood flow in skinned limb (B.F. Mus.), vascular resistance (V.R.) and mean and phasic blood pressure (B.P.). Time calibration, 10 sec.

3. It was found that the pressure–flow curve obtained during stimulation of the nose was shifted to the right of the two control curves obtained before and after the period of stimulation. The results obtained in one experiment are shown in Fig. 3*A* and were similar to those observed in a second experiment.

TABLE 2. The effects of stimulation of the nasal mucous membrane on the resistance of various vascular territories under conditions in which the arterial blood pressure or the blood flow was constant

Vascular territory	No. of expts.	Nos. of tests	Mean arterial blood pressure (mm Hg)		Blood flow (ml./min)		Vascular resistance		
			C	Change	*C*	Change	*C* (mm Hg/ml. min)	Change (mm Hg/ml. min)	Change (%)
Intact lower limb	9	24	133·0±3·3 (100–160)	0	64·5±5·6 (35–110)	−32·9±4·0 (−5 to −75)	2·47±0·21 (0·91–4·57)	2·88±0·54 (0·62–9·77)	122·2±20·2 (17–358)
Intact lower limb	1	4	133·5±4·0 (125–150)	26·5±4·1 (8–36)	Constant		—	—	19·8±3·2 (6–26)
Lower limb (muscle)	1	4	152·0±0·8 (150–154)	0	35·0±3·5 (30–45)	−25·2±5·0 (−15 to −37)	4·46±0·40 (3·34–5·06)	14·1±4·60 (5·74–26·4)	337·7±116·5 (113–600)
Lower limb paw (skin)	2	6	130·2±1·4 (125–136)	0	6·1±0·4 (5·0–8·0)	−1·4±0·4 (−1·0 to −1·8)	21·7±1·4 (16·2–26·0)	7·13±1·18 (3·2–10·1)	32·0±4·3 (16–45)
Common carotid	1	4	128·7±1·3 (125–130)	0	126·2±2·4 (120–130)	17·2±4·4 (10–30)	1·02±0·01 (1·00–1·04)	−0·12±0·03 (−0·07 to −0·21)	−12·0±2·8 (−7 to −20)
Vertebral	1	4	131·0±1·3 (128–134)	0	72·5±6·6 (54–85)	−33·7±2·4 (−30 to −40)	1·85±0·18 (1·57–2·36)	1·97±0·72 (0·99–4·14)	100·0±25·7 (60–175)
Splenic	2	5	124·0±2·4 (120–130)	0	78·0±7·3 (60–100)	−21·0±5·9 (−11 to −40)	1·64±0·14 (1·30–2·00)	0·56±0·13 (0·36–1·07)	37·4±11·4 (20–80)

The open values are the means ±S.E. of mean; those in parentheses the range.

It is concluded from these experiments that the increase in vascular resistance is due to vasoconstriction.

Skinned limb. To define more clearly the site of the increased vascular resistance occurring in the intact limb, three experiments were carried out on the skinned limb to determine the response of muscle vessels. The results are summarized in Table 1 showing that stimulation of the nasal mucous membrane caused a mean reduction in muscle blood flow of

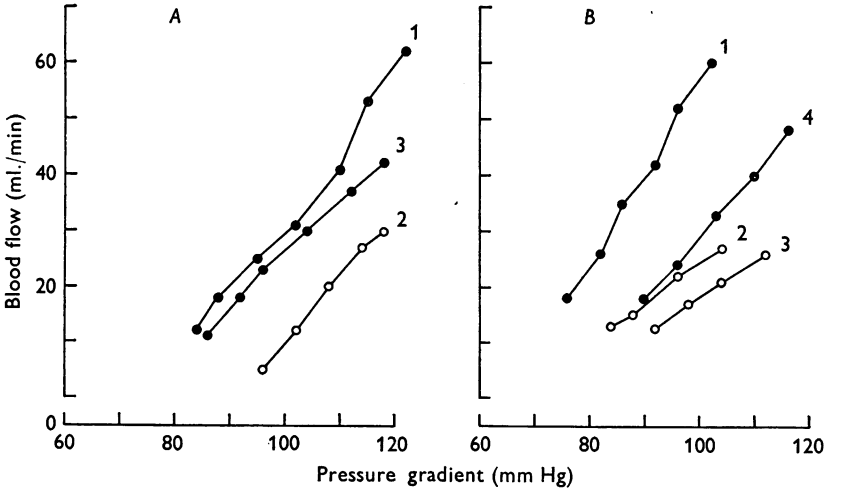

Fig. 3. The effects of stimulation of the nasal mucous membrane on the relationship between the pressure gradient (arterial blood pressure minus venous pressure) and femoral arterial blood flow in the intact limb (*A*) and skinned limb (*B*). Collateral blood flow abolished by tying a tape round the upper part of the legs. *Filled circles* (●), control curves; *open circles* (○), curves obtained during stimulation of the nose. The curves are numbered according to the order in which they were obtained.

$58{\cdot}4 \pm 6{\cdot}9\,\%$ and an increase in calculated vascular resistance of $222{\cdot}3 \pm 52{\cdot}6\,\%$ ($P < 0{\cdot}005$). Similar changes in vascular resistance were obtained when the blood pressure was constant (Table 2). It may be noted in Fig. 2 that the time course of the change in vascular resistance in the skinned limb is similar to that measured simultaneously in the opposite intact lower limb.

As with the intact limb stimulation of the nasal mucosa caused a shift to the right in the position of the pressure–flow curve (Fig. 3*B*).

Lower-limb paw. The evidence presented in Table 1 indicates that stimulation of the nasal mucous membrane also caused an increase in vascular resistance in the paw of $32{\cdot}5 \pm 3{\cdot}6\,\%$ ($P < 0{\cdot}001$). It also increased in tests in which there was no change in arterial blood pressure (Table 2).

Common carotid artery. In contrast to the change in blood flow in the

femoral artery elicited by stimulation of the nasal mucous membrane, the changes in flow in the common carotid artery were relatively smaller and tended to vary with the arterial blood pressure (Table 1; Fig. 7*A*). In twenty-eight tests in eight experiments, there was a mean reduction in arterial blood pressure of $6{\cdot}5 \pm 3{\cdot}0$ %, and in blood flow of $2{\cdot}7 \pm 6{\cdot}1$ %. The carotid vascular resistance increased $1{\cdot}8 \pm 4{\cdot}1$ % which is not statistically significant ($P > 0{\cdot}6$, Table 1). There was a decrease in carotid resistance in one experiment in which the arterial pressure remained constant (Table 2).

Vertebral artery. In seventeen tests in five experiments, the vertebral blood flow decreased in all except one test in one experiment in which no change took place. The calculated vascular resistance either increased (thirteen tests in four experiments), decreased (two tests in two experiments) or remained unchanged (two tests in two experiments). In all tests there was a mean increase in vascular resistance of $38{\cdot}3 \pm 9{\cdot}5$ % ($P < 0{\cdot}001$, Table 1)

Superior mesenteric artery. In all seven tests in three experiments there was a reduction in blood flow of $23{\cdot}4 \pm 4{\cdot}8$ % and in vascular resistance of $47{\cdot}6 \pm 12{\cdot}6$ % ($P < 0{\cdot}01$, Table 1). There was also a reduction in vascular resistance at constant blood pressure (Table 2).

Splenic artery. Stimulation of the nasal mucous membrane on five occasions in two experiments resulted, in an increase in calculated vascular resistance of $30{\cdot}8 \pm 8{\cdot}2$ % ($P < 0{\cdot}02$, Table 1). In the same two experiments, the vascular resistance also increased under conditions of constant pressure (Table 2).

Renal artery. The calculated vascular resistance increased by $22{\cdot}6 \pm 2{\cdot}8$ % in fifteen tests in four experiments on stimulation of the nose ($P < 0{\cdot}001$, Table 1).

Relation to changes in blood pressure

There was no consistent relationship between the change in arterial blood pressure and the change in vascular resistance in the intact limb (Fig. 4*A*), skinned limb (Fig. 4*B*, ○) or paw (Fig. 4*B*, ▲), or in the vascular beds of the vertebral artery (Fig. 5*A*, ○), mesenteric artery (Fig. 5*B*, ▲), splenic artery (Fig. 5*B*, △), or renal artery (Fig. 5*B*, □). The changes in vascular resistance cannot therefore be explained wholly on the basis of a reflex secondary to the change in pressure in the arterial baroreceptor areas. The same conclusion can be drawn from experiments on the carotid circulation (Fig. 5*A*, ● and Table 2).

Relation to changes in arterial P_{O_2} and P_{CO_2}

In eighteen experiments samples of arterial blood taken during spontaneous respiration had a mean P_{O_2} 407·8 ± 44·9 mm Hg (range 302–452), P_{CO_2} 44·9 ± 0·9 mm Hg (range 39–53) and pH 7·318 ± 0·01 (range 7·27–7·42). In seven of these blood was sampled at the end of the apnoeic period produced by stimulation of the nasal mucous membrane. Compared with the

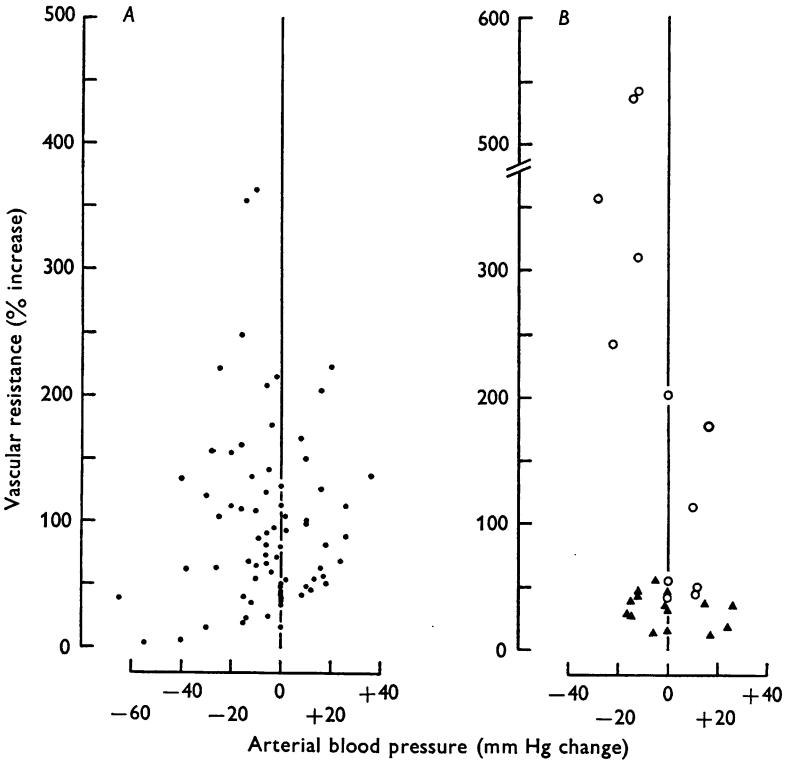

Fig. 4. The effects of stimulation of the nasal mucous membrane on the relationship between the change in mean arterial blood pressure and the change in vascular resistance in the intact limb, skinned limb and paw. *A*, intact limb. *B*, *open circles* (○), skinned limb; *filled triangles* (▲), paw. The numbers of tests and experiments correspond to those given in Table 1.

control samples, the P_{O_2} was reduced by 20·4 ± 13·7 mm Hg the P_{CO_2} was raised 4·4 ± 0·6 mm Hg and the pH was lowered by 0·027 ± 0·004.

To exclude the possibility that the observed changes in vascular resistance were secondary to the alterations in arterial blood P_{O_2}, P_{CO_2} and pH occurring during the inhibition of breathing, tests of stimulation of the nasal mucous membrane were carried out while respiration was main-

tained constant artificially. In three experiments it was found that there were no appreciable differences in the cardio-inhibitory and limb vasomotor responses elicited by stimulation of the nasal mucous membrane during spontaneous respiration and during artificial respiration.

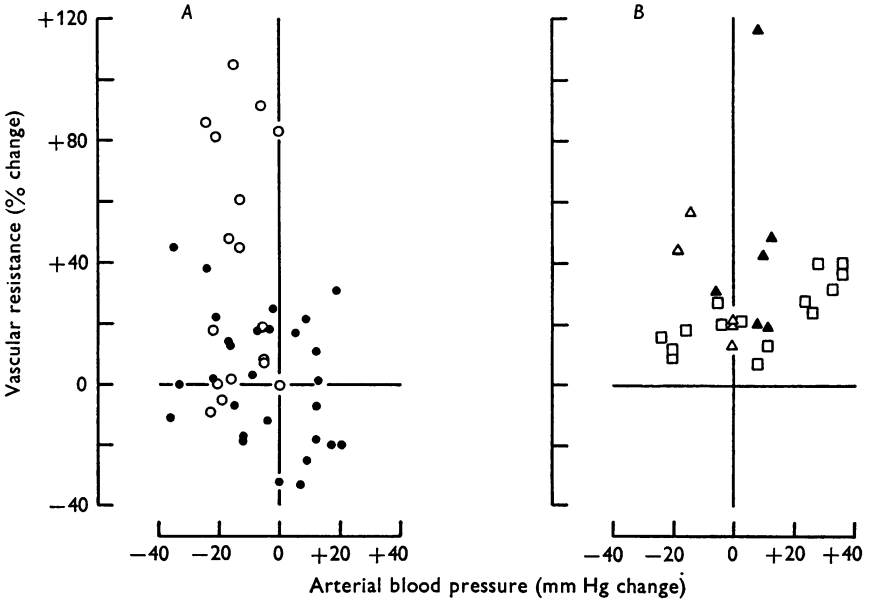

Fig. 5. The effects of stimulation of the nasal mucous membrane on the relationship between the change in mean arterial blood pressure and the change in vascular resistance in the vascular beds of the vertebral, common carotid, mesenteric, splenic and renal arteries. *A*, *filled circles* (●), common carotid artery; *open circles* (○), vertebral artery. *B*, *filled triangles* (▲), mesenteric artery; *open triangles* (△), splenic artery; *open squares* (□), renal artery. The numbers of tests and experiments correspond to those given in Table 1.

Withdrawal of the stimulus

Cessation of the stimulus nearly always caused a period of hyperventilation lasting up to 1 min before respiration returned to its original level (Figs. 2, 6, 7, 8*A* and 9).

In twelve of thirty experiments the heart rate and blood pressure gradually returned to normal after cessation of the stimulus. In eighteen experiments (thirty-nine observations), however, there was an immediate increase in heart rate and blood pressure to levels exceeding their control values. The heart rate rose 29·9 ± 4·0 beats/min (range 5–82) and the mean blood pressure 34·5 mm Hg (range 2–100) above the resting level before returning to normal. These values represent increases of 33·7 ± 4·5 and 28·2 ± 2·2 % respectively (Figs. 2 and 7*A*).

In nine experiments (twenty-one observations) there was an after-vasodilatation in the femoral vascular bed, the vascular resistance falling by 43·0 ± 5·9 % (range 0–79) of the control value (Fig. 2). In one out of three experiments carried out on a skinned limb, a reduction in vascular resistance of 72 % was observed (average of three tests). This experiment is illustrated by Fig. 2. These vascular responses persisted for up to 2 min. No consistent after-effects were observed in other vascular beds.

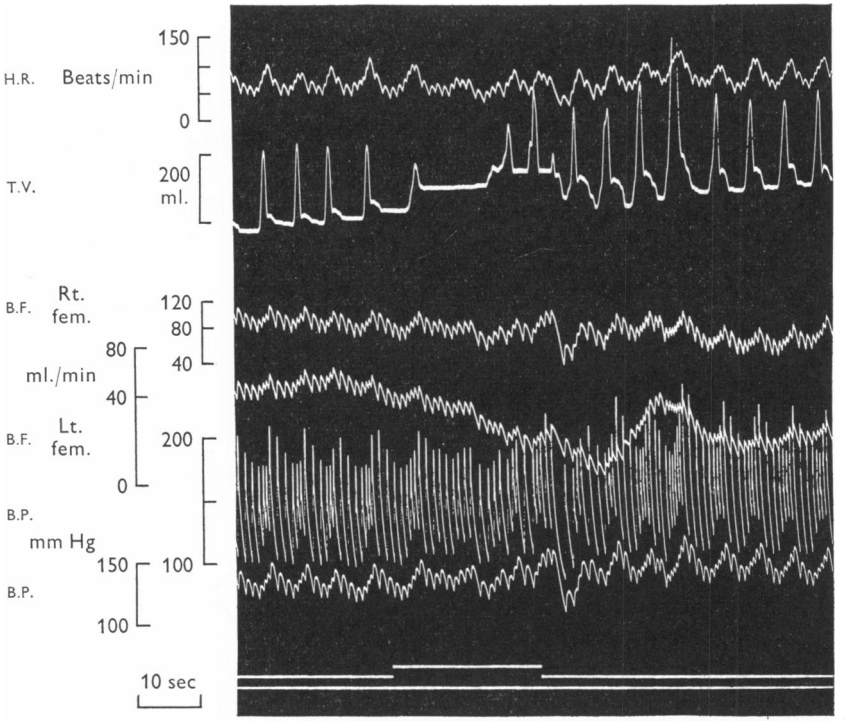

Fig. 6. Effects of stimulation of the nasal membrane by water at 21° C on the normal and denervated limb blood flows. Dog, male, 14·7 kg. Morphine–chloralose-urethane anaesthesia. The right limb was denervated. Records from above downwards; heart rate (H.R.), tidal volume (T.V.), right femoral blood flow (denervated limb, B.F. Lt. fem.), left femoral blood flow (innervated limb, B.F. Rt. fem.), blood pressure (B.P.) and mean blood pressure (B.P.). Time calibration, 10 sec.

Reflex nature of the responses

Afferent nerve pathways. The application of procaine hydrochloride (2·5 %) to the nasal mucous membrane invariably abolished the respiratory, cardiac and vasomotor responses (six experiments). All the responses reappeared 30–60 min later when the effects of the local anaesthetic had worn off.

In five experiments the effects of cutting branches of the trigeminal nerves on the respiratory and cardiovascular responses were studied. It was found that the responses were diminished in size by dividing either the ethmoidal nerves or the maxillary branch of the trigeminal nerves. Only when both nerves were divided on both sides were the responses abolished.

Efferent nerve pathways. Division of both cervical vagosympathetic nerves in three experiments considerably diminished or abolished the cardio-inhibitory response resulting from stimulation of the nasal mucous membrane. The blood pressure now usually increased, but the respiratory and vascular responses were relatively unaffected.

With regard to the efferent pathway mediating the vascular responses, it was found that division of the femoral and sciatic nerves abolished the vasoconstriction occurring in the lower limb (three experiments). Division of the nerves to an isolated perfused upper limb also abolished the vasoconstrictor response (one experiment). In two further experiments, one lower limb was denervated before recording began, and blood flow was measured simultaneously in both femoral arteries. The typical responses occurring in one of these experiments is illustrated in Fig. 6. Stimulation of the nose had little or no effect on mean arterial pressure or on femoral blood flow to the denervated limb so that the calculated vascular resistance remained unchanged. The blood flow to the innervated limb, however, decreased from 45 to 4 ml./min representing an increase in vascular resistance of 464%.

Infiltration of procaine hydrochloride (2%) around the ankle joint also abolished the increased vascular resistance which occurred in the perfused paw.

Action of drugs

Atropine. In eight experiments, intravenous atropine, 0·2 mg/kg, diminished or abolished the cardio-inhibitory response occurring as a result of stimulation of the nasal mucosa, but did not appreciably affect the vascular resistance changes occurring in any of the vascular territories tested (Fig. 7*A*, *B*). The after-vasodilatation occurring in the intact limb was also unaffected.

Propranolol. This β-receptor adrenergic blocking agent was used in doses of 0·5–1·0 mg/kg and had no effect on the cardiac or immediate vascular responses to nasal stimulation (three experiments). In two of these experiments, however, propranolol considerably reduced the after-rise in whole-limb and muscle blood flow and the reduction in vascular resistance which was conspicuous in tests before the β-blocker was given.

Hexamethonium, bretylium tosylate. The effects of the ganglionic blocking agent hexamethonium (10 mg/kg) and the adrenergic blocking drug

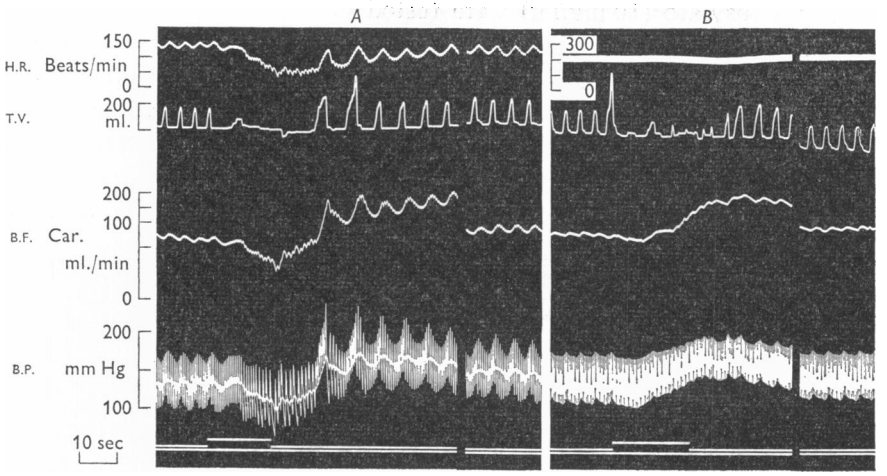

Fig. 7. Effects of stimulation of the nose by water at 21° C before (*A*) and after (*B*) atropine, 2 mg intravenously. Dog, female, 13·3 kg. Morphine–chloralose-urethane anaesthesia. Records from above downwards: heart rate (H.R.), tidal volume (T.V.), right common carotid blood flow (B.F. Car.), and mean and phasic blood pressure (B.P.). During the break in the records in *A*, a period of 30 sec elapsed. Time calibration, 10 sec.

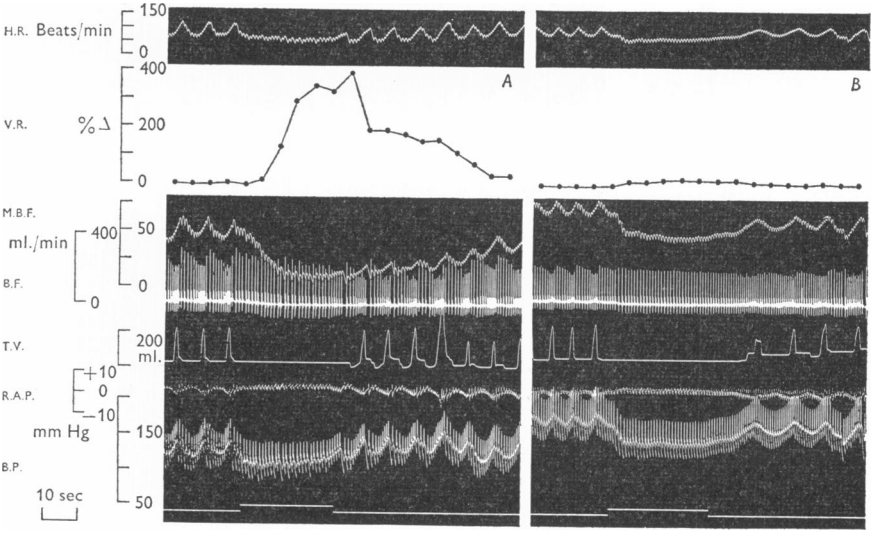

Fig. 8. The effects of stimulation of the nasal mucous membrane by water at 22° C before (*A*) and after (*B*) bretylium tosylate, 10 mg/kg. Dog, male, 16·7 kg. Morphine–chloralose-urethane anaesthesia. The insert shows the percentage increase in calculated vascular resistance in the normal lower limb. Records from above downwards: heart rate (H.R.), femoral vascular resistance (V.R.), mean (M.B.F.) and phasic (B.F.) femoral blood flow, tidal volume (T.V.), right atrial pressure (R.A.P.), and mean and phasic arterial blood pressure (B.P.). Time calibration, 10 sec.

bretylium tosylate (10 mg/kg) were tested on the vascular responses to stimulation of the nasal mucous membrane. In seven experiments it was found that the increase in vascular resistance occurring in the intact limb, skinned limb and paw, and in the superior mesenteric, splenic and vertebral vascular beds were all abolished. The effects of bretylium tosylate in one experiment are shown in Fig. 8*A*, *B*.

Neither of these drugs affected the respiratory responses (Fig. 8*A*, *B*).

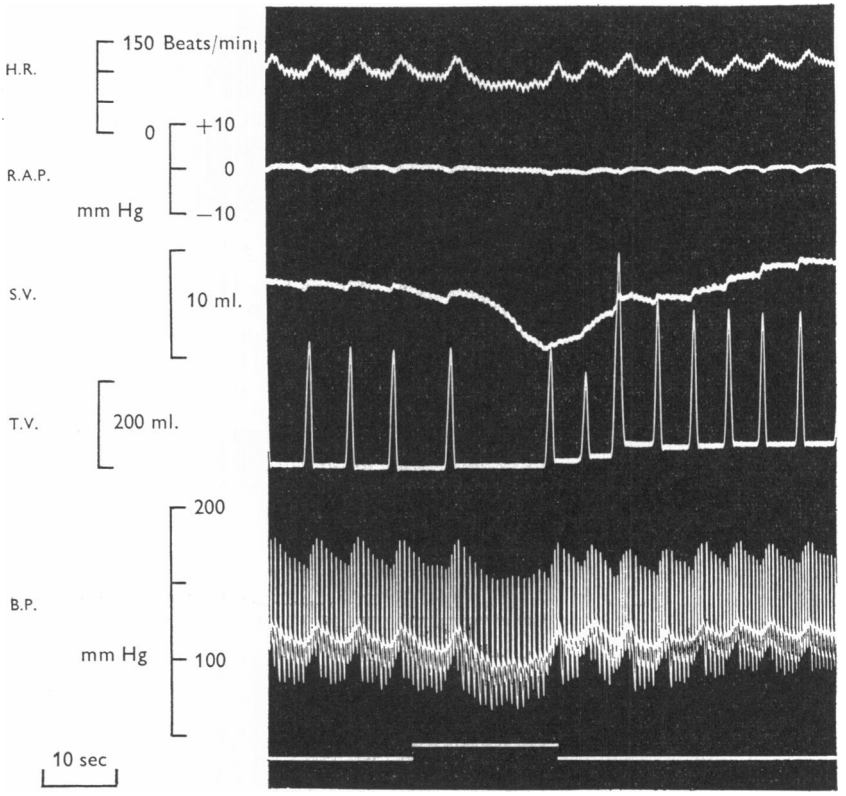

Fig. 9. Effects of stimulation of the nasal mucous membrane by water at 21° C on volume of the spleen. Dog, female, 12·3 kg. Morphine–chloralose-urethane anaesthesia. Records from above downwards: heart rate (H.R.), mean right atrial pressure (R.A.P.), splenic volume (S.V.), tidal volume (inspiration upwards) (T.V.), mean and phasic arterial blood pressure (B.P.). Time calibration, 10 sec.

Changes in volume of the spleen

Stimulation of the nasal mucous membrane in thirteen tests in four experiments caused a reduction in volume of the spleen of $5{\cdot}3 \pm 1{\cdot}7$ ml. (range 3–12; $P < 0{\cdot}01$) (Fig. 9). In one experiment, the response was

abolished by local anaesthesia of the nasal mucosa, and in another it was abolished by hexamethonium, 10 mg/kg I.V.

Effects of changes in temperature of nasal perfusate

The cardiac and intact limb vascular responses were compared in tests in which the temperature of the water perfusing the nose was 3 or 10° C, 22–23° C and 37–39° C. In six such experiments there were no consistent differences in the responses elicited at the different temperatures. The cardio-inhibitory responses at a nasal perfusion temperature of 37–39° C were the same as those at the two lower temperatures in five experiments, but greater in the remaining experiment. With the vascular responses, the increase in resistance occurring at a nasal perfusion temperature of 37–39° C was larger (one experiment), or smaller (one experiment) than the response produced by the perfusate at the two lower temperatures; in the remaining four experiments, there was no difference.

DISCUSSION

We have confirmed previous observations that stimulation of the nasal mucous membrane causes apnoea in the expiratory position and bradycardia (Kratschmer, 1870; Brodie & Russell, 1900; Dixon & Brodie, 1903; Allen, 1928). The blood pressure responses are variable not only between animals of the same species but also between animal species. Thus, whereas the predominant effect in the dog is a small fall in pressure, that in the rabbit is a conspicuous rise in pressure in response to stimulation of the nasal mucous membrane by irritant vapours and by water (Allen, 1928; Ebbecke & Knüchel, 1943). We have shown further that these effects are accompanied by reflex changes resistance in some vascular territories and by a small contraction of the spleen. Vasoconstriction takes place in the intact limb, in skin and muscle, and in the vascular beds of the superior mesenteric, splenic, vertebral and renal arteries. No change in vascular resistance occurred in the common carotid circulation.

These responses are reflex in nature, being abolished by division of the trigeminal nerves (Kratschmer, 1870; Allen, 1928; the present paper). The receptors concerned are most likely the free nerve-endings in the nasal respiratory mucosa which are all of one kind. They are simple in structure, lie within the lamina propria or on the epithelium and are derived from non-myelinated axons (Cauna, Hinderer & Wentges, 1969). The question as to whether the same receptors are responsible for the reflex hyperpnoea, which sometimes occurs on subjecting the nasal mucosa to ammonia vapour is uncertain.

We did not find that the temperature of the water or saline perfusing

the nose had any consistent effect on the duration of the apnoea, bradycardia or the vasoconstrictor response in the intact hind limb. The temperature of the water into which the head of an animal is submerged is also without effect on the cardio-inhibitory response (duck: Butler & Jones, 1968; rabbit, sheep, new-born lamb: Tchobroutsky, Merlet & Rey, 1969). In man, however, the bradycardia of *face* immersion is temperature dependent, the size of the response increasing as the temperature of the water is reduced (Whayne & Killip, 1967; Kawakami, Natelson & DuBois, 1967; Corriol & Rohner, 1968; Song, Lee, Chung & Hong, 1969).

With regard to the vasoconstrictor responses elicited by stimulation of the nasal mucous membrane, it was important to establish whether they were due to a primary or direct reflex from the nose or were secondary to some other effect. It was established that they persisted during controlled ventilation and could not therefore be attributed to changes in arterial blood P_{O_2} and P_{CO_2} or to a pulmonary vagal reflex through cessation of lung movements in the expiratory position (Daly, Hazzledine & Ungar, 1967; Daly & Robinson, 1968). A rise in arterial blood pressure sometimes occurred but as there was no consistent relation between blood pressure and the change in vascular resistance, it is unlikely that the vasoconstrictor responses can be attributed wholly to an arterial baroreceptor reflex. It is also unlikely that they are due to the reflex secretion of suprarenal catecholamines (Allison & Powis, 1971) for two reasons. First, the onset of the response occurred before the catecholamines could have reached the vascular bed, particularly in those experiments involving an extracorporeal circulation. And, secondly, denervation of the vascular bed invariably abolished the response. The most likely explanation of the responses is therefore that they are the result of a primary reflex from the nose.

The slowing of the heart is largely vagal in origin since the response was reduced or abolished by division of the vagus nerves or by atropine. Evidence is presented that the vasoconstrictor responses are mediated by sympathetic adrenergic nerves. Daly & Scott (1961) showed that electrical stimulation of post-ganglionic sympathetic fibres in the splenic nerve caused a reduction in volume of the spleen of the dog and an increase in splenic arterial vascular resistance under conditions of controlled perfusion of the organ with autologous blood. Similar responses occur during a stimulation of the nasal mucous membrane, which being abolished by hexamethonium, are presumably mediated by the sympathetic nervous system (present paper). These reflexly induced responses, however, differed from those evoked by electrical stimulation of the splenic nerve observed by others (Daly & Scott, 1961; Davies, Gamble & Withrington, 1968) in that the volume changes were small by comparison with vascular effects. The reason for this is not at present clear, but the possibility that

the nasal reflex produces a selective effect on central sympathetic neurones supplying the capsular smooth muscle and blood vessels of the spleen cannot be excluded.

Stimulation of receptors in and around the nose is believed to be an important mechanism responsible for the cardiovascular adaptations occurring in marine mammals during submersion in water (see Anderson, 1966; Angell James & Daly, 1972). Intense peripheral vasoconstriction occurs during diving and although the cardiac output is drastically reduced, the cerebral and coronary blood flows are maintained so that the limited supplies of oxygen are preferentially directed to the brain and heart muscle. On the evidence presented in the present paper, it is not possible to deduce the changes in cerebral blood flow that occur during stimulation of the nose. The common carotid vascular resistance did not change, but the change in partition of flow between the various branches of the common carotid arteries is not known. In the dog brain receives a carotid blood supply not only by the internal carotid artery, but also the external carotid through the anastomotic branch of the artery (Bouckaert & Heymans, 1935; Chungcharoen, Daly, Neil & Schweitzer, 1952). The reduction in blood flow and increase in vascular resistance in the vertebral artery could be accounted for by constriction of vessels in territories, particularly muscle, supplied by its branches rather than in the brain. The control of cerebral blood flow by this nasal reflex requires therefore further study.

Post-stimulus responses. In some experiments the reflex vasoconstriction occurring as a result of stimulation of the nasal mucous membrane was followed by an after-vasodilatation lasting up to 2 min. Although no detailed analysis of the mechanisms underlying this response was made, it was found to be unaffected by atopine and is unlikely therefore to be due to activation of sympathetic cholinergic vasodilator fibres. As the response was reduced by propranolol, a reflex secretion of suprarenal medullary catecholamines (Allison & Powis, 1971) could be involved. We have not, however, made a study of other possible mechanisms such as a local accumulation of metabolites, or a secondary response resulting in a reduction of activity in sympathetic vasoconstrictor fibres and engendered by the concomitant hyperventilation and rise of blood pressure.

The hyperventilation is probably the result of the increase in arterial blood P_{CO_2} occurring during the period of the apnoea (Angell James & Daly, 1969). So far as the cardiac and blood pressure effects are concerned, these responses are similar to those which occur as a result of withdrawal of the stimuli resulting from diving. Some of the mechanisms underlying these changes have been discussed elsewhere (Angell James & Daly, 1972). These post-stimulation responses, and in particular the large rise in blood

pressure, could have a clinical implication in man, for they may play an important part in the aetiology of cardiovascular accidents that occur during bathing.

Possible functions of reflex. A striking feature of the present study was the potency of the respiratory and cardiovascular responses elicited by stimulation of the nasal mucous membrane in the anaesthetized animal. Some of the circumstances under which the reflex is elicited in man have been discussed elsewhere (Angell James & Daly, 1969). The reflex appears to have two functions: first, it serves as a protective reflex preventing fluids and irritant vapours gaining access to the lungs, and secondly, it has a purposeful function in that it plays an important role in the initiation of cardiovascular adaptations occurring in diving mammals and aquatic birds (Angell James & Daly, 1969, 1972; Daly 1972).

Tchobroutsky *et al.* (1969) have suggested that the inhibition of respiratory movements of the foetus *in utero* is due in part to a reflex initiated by immersion of the head and particularly the contract of fluid with the glottis. They believe that at birth release of this inhibitory reflex helps to ensure the onset of breathing. Reflex respiratory and cardiovascular responses from the nasal mucous membrane could also participate at birth providing conditions are such that movement of the nasal fluid takes place (present paper).

We wish to express our thanks to Mr D. R. Bacon for expert technical assistance. This work was supported in part by grants from the Royal Society and St Bartholomew's Hospital Endowment Fund.

REFERENCES

ALLEN, W. F. (1928). Effect on respiration, blood pressure, and carotid pulse of various inhaled and insufflated vapors when stimulating one cranial nerve and various combinations of cranial nerves. I. Branches of the trigeminus affected by these stimulants. *Am. J. Physiol.* **87**, 319–325.

ALLEN, W. F. (1936). Studies on the level of anaesthesia for the olfactory and trigeminal respiratory reflexes in dogs and rabbits. *Am. J. Physiol.* **115**, 579–587.

ALLISON, D. J. & POWIS, D. A. (1971). Adrenal catecholamine secretion during stimulation of the nasal mucous membrane in the rabbit. *J. Physiol.* **217**, 327–339.

ANDERSON, H. T. (1966). Physiological adaptations in dividing vertebrates. *Physiol. Rev.* **46**, 212–243.

ANGELL JAMES, J. E. & DALY, M. DE B. (1969). Nasal reflexes. *Proc. R. Soc. Med.* **62**, 1287–1293.

ANGELL JAMES, J. E. & DALY, M. DE B. (1972). Some mechanisms involved in the cardiovascular adaptations to diving. *Soc. Exp. Biol. Symp.* (in the Press).

BACON, D. R., DALY, M. DE B. & SCOTT, M. J. (1962). A method for continuously recording respiration quantitatively during administration of various gas mixtures in the cat. *J. Physiol.* **161**, 2–3*P*.

BOUCKAERT, J. J. & HEYMANS, C. (1935). On the reflex regulation of the cerebral blood flow and the cerebral vaso-motor tone. *J. Physiol.* **84**, 367–380.

BRODIE, T. G. & RUSSELL, A. E. (1900). On reflex cardiac inhibition. *J. Physiol.* **26**, 92–106.

BUTLER, P. J. & JONES, D. R. (1968). Onset of and recovery from diving bradycardia in ducks. *J. Physiol.* **196**, 255–272.

CASSIN, S., DAWES, G. S., MOTT, J. C., ROSS, B. B. & STRANG, L. B. (1964). The vascular resistance of the foetal and newly ventilated lung of the lamb. *J. Physiol.* **171**, 61–79.

CAUNA, N., HINDERER, K. H. & WENTGES, R. T. (1969). Sensory receptor organs of the human nasal respiratory mucosa. *Am. J. Anat.* **124**, 187–210.

CHUNGCHAROEN, D., DALY, M. DE B., NEIL, E. & SCHWEITZER, A. (1952). The effect of carotid occlusion upon the intrasinusal pressure with special reference to vascular communications between the carotid and vertebral circulations in the dog, cat and rabbit. *J. Physiol.* **117**, 56–76.

CORBETT, L. J., KERR, J. H. & PRYS-ROBERTS, C. (1969). Cardiovascular responses to aspiration of secretions from the respiratory tract in man. *J. Physiol.* **201**, 51–52*P*.

CORRIOL, J. & ROHNER, J. J. (1968). Rôle de la température de l'eau dans la bradycardia d'immersion de la face. *Archs Sci. physiol.* **22**, 265–274.

DALY, M. DE B. (1972). Interaction of cardiovascular reflexes. *Lect. scient. Basis Med.* (in the press).

DALY, M. DE B., HAZZLEDINE, J. L. & UNGAR, A. (1967). The reflex effects of alterations in lung volume on systemic vascular resistance in the dog. *J. Physiol.* **188**, 331–351.

DALY, M. DE B. & ROBINSON, B. H. (1968). An analysis of the reflex systemic vasodilator response elicited by lung inflation in the dog. *J. Physiol.* **195**, 387–406.

DALY, M. DE B. & SCOTT, M. J. (1961). The effects of acetylcholine on the volume and vascular resistance of the dog's spleen. *J. Physiol.* **156**, 246–259.

DALY, M. DE B. & UNGAR, A. (1966). Comparison of the reflex responses elicited by stimulation of the separately perfused carotid and aortic body chemoreceptors in the dog. *J. Physiol.* **182**, 379–403.

DAVIES, B. N., GAMBLE, J. & WITHRINGTON, P. G. (1968). The separation of the vascular and capsular smooth muscle responses to sympathetic nerve stimulation in the dog's spleen. *J. Physiol.* **196**, 42–43*P*.

DIXON, W. E. & BRODIE, T. G. (1903). Contributions to the physiology of the lungs. Part I. The bronchial muscles, their innervation, and the action of drugs upon them. *J. Physiol.* **29**, 97–173.

EBBECKE, U. & KNÜCHEL, F. (1943). Über den Trigeminus-Atem-Schluck- und Herzreflex beim Kaninchen. *Pflügers Arch. ges. Physiol.* **247**, 255–263.

FRANÇOIS-FRANCK, C. A. (1889). Contribution à l'étude expérimentale des névroses réflexes d'origine nasale. *Archs Physiol.* **21**, 538–555.

FRANK, O. (1903). Kritik der elastischen Manometer. *Z. Biol.* **44**, 445–613.

GADDUM, J. H. & KWIATKOWSKI, H. (1938). The action of ephedrine. *J. Physiol.* **94**, 87–100.

GESSNER, U. & BERGEL, D. (1964). Frequency response of electromagnetic flowmeters. *J. appl. Physiol.* **19**, 1209–1211.

KAWAKAMI, Y., NATELSON, B. H. & DUBOIS, A. B. (1967). Cardiovascular effects of face immersion and factors affecting diving reflex in man. *J. appl. Physiol.* **23**, 964–970.

KRATSCHMER, F. (1870). Über Reflexe von der Nasenschleimhaut auf Athmung und Kreislauf. *Sber. Akad. Wiss. Wien* **62**, 147–170.

LINDGREN, P. (1958). An improved method for drop recording of arterial and venous blood flow. *Acta physiol. scand.* **42**, 5–11.

SONG, S. H., LEE, W. K., CHUNG, Y. A. & KONG, S. K. (1969). Mechanism of apneic bradycardia in man. *J. appl. Physiol.* **27**, 323–327.

TCHOBROUTSKY, C., MERLET, C. & REY, P. (1969). The diving reflex in rabbit, sheep and newborn lamb and its afferent pathways. *Resp. Physiol.* **8**, 108–117.

WHAYNE, T. F., JR. & KILLIP, T., III. (1967). Simulated diving in man: Comparison of facial stimuli and response in arrhythmia. *J. appl. Physiol.* **22**, 800–807.