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THE PATTERN OF CARDIOVASCULAR RESPONSE TO CAROTID CHEMORECEPTOR STIMULATION IN THE CAT

BY S. M. HILTON AND J. M. MARSHALL

From the Department of Physiology, The Medical School, University of Birmingham, Birmingham B15 2TJ

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SUMMARY

1. The pattern of cardiovascular response evoked by carotid chemoreceptor stimulation has been investigated in cats anaesthetized by continuous infusion of Althesin (Glaxo).

2. A variety of chemoreceptor stimulants, injected retrogradely into the lingual artery with the external carotid artery ligated, evoked hyperventilation with variable changes in arterial pressure and heart-rate, but a consistent vasodilatation in limb muscles and vasoconstriction in renal, mesenteric and cutaneous vasculature.

3. The muscle vasodilatation was still obtained after vagotomy and when the animal was paralysed and artificially ventilated; thus, it was not secondary to the hyperventilation.

4. In the majority of experiments the muscle vasodilatation was much reduced or abolished by atropine indicating it was mediated by sympathetic cholinergic fibres, which is characteristic of the alerting stage of the defence reaction in the cat. The cardiovascular pattern was accompanied by the other autonomic features of the alerting response, viz. pupillary dilatation, retraction of the nictitating membranes and pilo-erection.

5. In one and the same animal the pattern of response evoked by carotid chemoreceptor stimulation was the same as that evoked by noxious cutaneous stimulation, and by electrical stimulation in the brain stem defence areas.

6. It is concluded that peripheral chemoreceptor stimulation acts as an excitatory input to the hypothalamic and brain stem defence areas and that it can readily evoke the autonomic components of the alerting stage of the defence reaction. It is suggested that this has been missed in previous studies on anaesthetized animals because of the depressant action of chloralose and barbiturates on transmission in the hypothalamus and mid-brain.

INTRODUCTION

It has become accepted that stimulation of peripheral chemoreceptors evokes bradycardia and peripheral vasoconstriction, but that these changes may be overcome in the spontaneously breathing animal by secondary effects resulting from the evoked hyperventilation, to give tachycardia and vasodilator responses, particularly in the

mesenteric vasculature and in skeletal muscle. These ideas stem mainly from work carried out by Daly and his co-workers on dogs anaesthetized with chloralose or barbiturates, their many careful investigations leading to the conclusion that these secondary effects comprise (*a*) reflexes initiated by pulmonary stretch receptors with afferent fibres coursing in the vagus (Daly & Scott, 1962), (*b*) central effects of reduced arterial P_{CO_2} secondary to hyperventilation (Daly & Scott, 1963) and (*c*) direct effects of increased central inspiratory drive (see Daly, 1972). Vasodilatation has also been seen in muscle and mesentery on cessation of chemoreceptor stimulation, even in artificially ventilated dogs, but this has been ascribed to post-stimulatory inhibition of chemoreceptor discharge (Bernthal & Schwind, 1945), reactive hyperaemia following the initial reflex vasoconstriction or to the effects of circulating catecholamines (Daly & Scott, 1962).

By comparison the effects of peripheral chemoreceptor stimulation in the anaesthetized cat are not so well documented. MacLeod & Scott (1964) found that chemoreceptor stimulation evoked bradycardia even when the animal was breathing spontaneously, suggesting that the tendency for hyperventilation to induce tachycardia was less marked than in the dog. More recently, Little & Öberg (1975) reported that cats which were paralysed, artificially ventilated and vagotomized showed a tachycardia on chemoreceptor stimulation. The responses in the peripheral vascular beds of these same animals were broadly similar to those reported by Daly & Scott (1962), for there was vasoconstriction in cutaneous, renal and mesenteric vasculature as well as in skeletal muscle.

In marked contrast to these results obtained in experiments on anaesthetized animals, Bizzi, Libretti, Malliani & Zanchetti (1961) showed that in the unanaesthetized cat, decerebrated so as to spare the hypothalamus, stimulation of carotid chemoreceptors evoked sham rage characterized by a dramatic rise in arterial blood pressure together with hyperventilation, dilatation of the pupils, retraction of the nictitating membranes, struggling and clawing movements, lashing of the tail and the facial expression of rage. When Hilton & Joels (1965) confirmed these observations, they reported that the response included tachycardia and an increase in muscle blood flow which were still present when the animal was paralysed and artificially ventilated. Although the muscle vasodilatation was not tested for atropine sensitivity which is a characteristic feature of the muscle vasodilatation of the defence reaction in the cat, these experiments, like those of Bizzi *et al.* (1961), indicated that in the high decerebrate cat at least peripheral chemoreceptor stimulation can activate the hypothalamic and brainstem defence areas.

A hypothesis which would draw these two sets of results together is that peripheral chemoreceptors provide an excitatory input to the defence areas, but that the full expression of the response is prevented by the depressant action of the commonly used anaesthetics on synaptic transmission in the central nervous system, particularly in the hypothalamus and mid-brain. Evidence for this proposal has come from previous studies which provide ample demonstration that chloralose and barbiturates, when given in the usual anaesthetic doses, have a blocking action on synapses in pathways afferent to the brain stem defence areas. Thus in cats anaesthetized with these agents neither electrical stimulation in the amygdaloid defence areas nor noxious cutaneous stimuli, both of which activate the brain stem defence areas

synaptically, will evoke the autonomic pattern of the alerting stage of the defence reaction (Abrahams, Hilton & Zbrozyna, 1960, 1964; Hilton & Zbrozyna, 1963). Timms (1976, 1981) recently showed that the steroid anaesthetic, Althesin (Glaxo), does not have the same depressant action as the more conventional anaesthetics. When given by continuous intravenous infusion in the cat, this agent produces full anaesthesia and yet still allows the autonomic components of the alerting response to be evoked by electrical stimulation in the amygdala. These findings suggested that experiments under Althesin anaesthesia might provide a more ideal background from which to study the effects of peripheral chemoreceptor stimulation.

The acceptance of the hypothesis that defence area activation is an integral component of the response to chemoreceptor stimulation must rest heavily on establishing that any muscle vasodilatation which has been produced is a direct consequence of chemoreceptor activity and is not secondary to hyperventilation. In fact, vasodilatation is evoked in muscle as part of the primary response to chemoreceptor stimulation in the Althesin-anaesthetized cat and it is mediated at least in part by cholinergic fibres, as has already been reported in a preliminary communication (Marshall, 1977).

METHODS

Experiments were performed on thirty-six female cats. Anaesthesia was induced with either ethyl chloride and halothane or nitrous oxide and oxygen (80%:20%) with halothane, and was maintained with a continuous intravenous infusion of Althesin (Glaxo Labs Ltd.), as described by Timms (1976). This agent was given at a rate of 8–16 mg total steroids/kg . hr during surgery and of 3–6 mg total steroids/kg . hr during the experimental period. The state of anaesthesia was judged by means of a fronto-occipital e.e.g., monitored via two stainless steel screws (10 BA) set in the cranium and displayed on an oscilloscope (Telequipment DM64) or pen recorder (Devices): it ranged from deep surgical anaesthesia during the preparatory period to a lighter level during the experiment itself when there was a hypersynchronous spindling e.e.g. with little burst suppression, the animal showing no spontaneous movements or brisk withdrawal reflexes (cf. Timms 1976, 1981).

Four high decerebrate preparations in which the hypothalamus is spared were prepared as described by Abrahams *et al.* (1960) by undercutting with a scalpel or by means of suction apparatus. In these experiments anaesthesia was maintained with halothane throughout the decerebration and other surgical procedures, after which it was discontinued.

Arterial blood pressure was recorded from a femoral artery via a pressure transducer (Bell & Howell) and heart-rate was derived from the pressure pulse by an instantaneous rate-meter (Miller, 1976). In each experiment, blood flow was recorded from a femoral artery (thirty-six experiments) and either the radial artery (two experiments), cranial mesenteric artery (twenty experiments) or the left renal artery (ten experiments), using cuff-type electromagnetic transducers (Biotronex Laboratory or Micron Instruments) which were calibrated *in vitro* using constant flow perfusion of a length of freshly obtained artery. The femoral artery was approached from the medial aspect and the probe placed distal to the deep femoral branch; the brachial artery also was approached from the medial aspect. In both cases the paw circulation was excluded by a stout ligature around the ankle or wrist. The renal artery was approached retroperitoneally and the mesenteric artery intraperitoneally, the probes being placed as close as possible to the dorsal aorta and care being taken to avoid damage to the renal or coeliac nerve plexus. A zero flow signal was obtained at regular intervals using an occluding snare placed distal to the flow transducer. The vascular conductance of each vascular bed was computed on-line using a custom-built electronic divider which made a beat-by-beat division of the arterial flow by the arterial blood pressure. In some experiments registrations were also made of changes in skin blood flow using an air-filled plethysmograph which was placed on a forepaw and connected to a volumetric pressure transducer (Grass PT5A). Respiration was monitored routinely both as tracheal air-flow using a pneumotachograph (Metabo

type 000) and, with the aid of Devices conditioning units, as tidal volume or, more often, as minute volume. All variables were displayed on pen recorders (Devices Ltd.).

The carotid chemoreceptors were stimulated with solutions of KCN, NaCN, Krebs solution or saline equilibrated with CO_2 or an isotonic mixture of NaH_2PO_4 and Na_2HPO_4 as described by Hilton, Spyer & Timms (1972) or isotonic NaH_2PO_4. All injections were made retrogradely through a cannula in the lingual artery with the external carotid artery ligated: at each test 0·3–0·5 ml. was delivered with a 1 ml syringe over a period of 10–20 sec.

In many experiments cutaneous nerve fibres were stimulated either via a needle inserted subcutaneously with an indifferent electrode nearby or by bipolar silver-cuff electrodes placed on the superficial radial nerve. Rectangular pulses of 0·3–0·5 msec duration were delivered at 40 Hz for periods of 5–10 sec. Peak-to-peak current strength was measured throughout stimulation as the voltage drop across a 1 kΩ resistor in series with the stimulating electrode and displayed on a storage oscilloscope (Telequipment, DM 53A) and was between 0·5 and 1·5 mA.

Muscular paralysis was achieved in some experiments with gallamine triethiodide (Flaxedil) given intravenously (3–4 mg/kg). Artificial respiration was then maintained at the level required to keep arterial blood pressure, pH, P_{CO_2} and plasma bicarbonate within the normal limits. For this purpose, arterial blood samples were taken routinely during experiments and analysed with Radiometer equipment. During the period of paralysis Althesin was infused continuously at the same rate as in the immediately preceding period. Adequate anaesthesia was maintained throughout as judged by the e.e.g., the stability of arterial blood pressure, heart-rate and regional blood flows and the fact that all these variables were within the range of values recorded in the absence of muscular paralysis.

RESULTS

The pattern of cardiovascular response to chemoreceptor stimulation

The pattern of response produced by stimulation of carotid chemoreceptors, in addition to the characteristic hyperventilation, always included pupil dilatation and retraction of the nictitating membrane, and sometimes pilo-erection. The changes in blood pressure and heart-rate were variable but there was consistently a vasodilatation in the fore- and hind-limb muscles preceded by a small constriction, and a constriction in the mesenteric and renal vascular beds. All of these features were seen with each of the test solutions: in practice the phosphate solutions, Krebs solution or saline equilibrated with CO_2 were used more frequently because they remained effective with repetition over many hours. The concentrations of the solutions were chosen for individual animals so as to produce a respiratory response of no more than 350% increase in minute volume over 1–2 min: they were 10–30% of the 'standard' strength in the case of the phosphate solutions, and 10–15 mg/ml. in the case of NaCN, KCN or DNP. Both the respiratory and cardiovascular changes induced by these agents were abolished after sectioning the ipsilateral sinus nerve and after ligation of the occipital and ascending pharyngeal arteries (J. M. Marshall, unpublished observations). This indicates that they were not due to any central action of the test solutions but rather were initiated by stimulation of the carotid chemoreceptor afferent fibres.

The respiratory changes began within the first two breaths after the onset of injection; respiratory rate and depth reached a peak within 10 sec and then gradually returned to control over the next 1–1½ min (Fig. 1). However, in some cases the hyperventilation was punctuated by a brief period of apnoea (usually inspiratory) which lasted 5–10 sec and began one to two breaths after beginning the injection.

Usually, arterial blood pressure showed an initial rise followed by a fall which coincided with the peak of the muscle vasodilatation (Fig. 1). Sometimes, however,

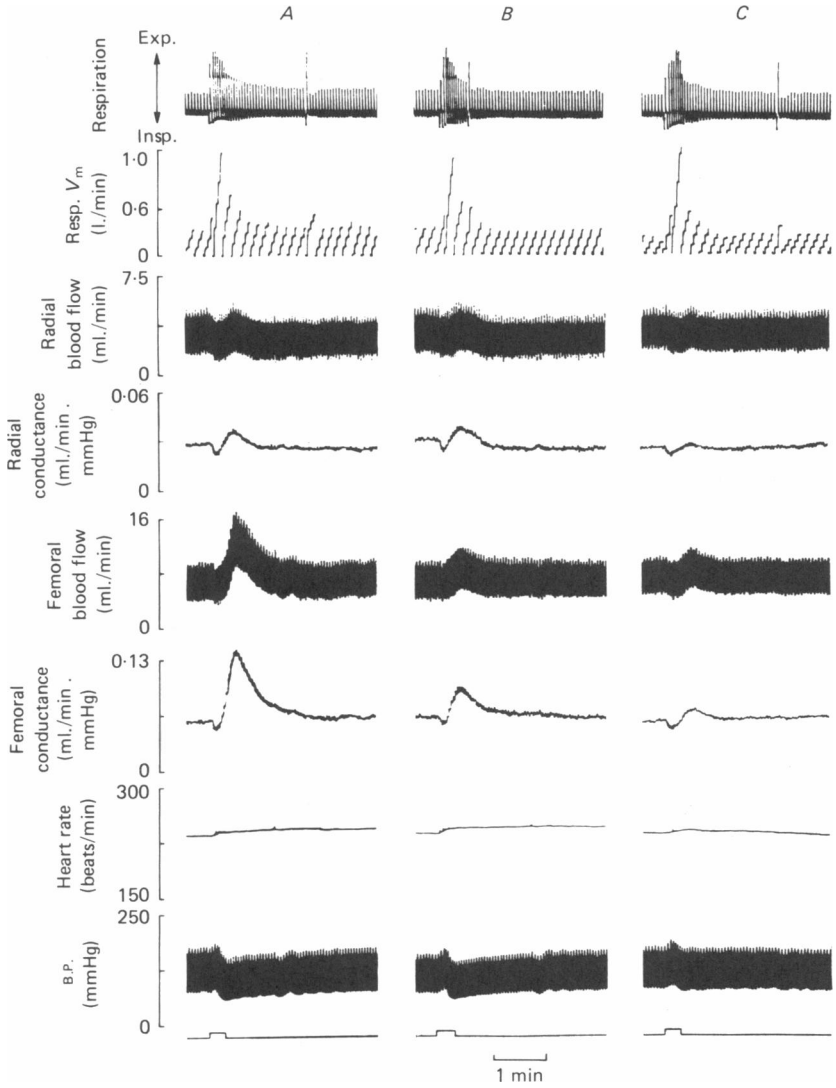

Fig. 1. Cat, Althesin. Effect of atropine on the responses evoked on stimulation of carotid chemoreceptors with inorganic phosphate solution. Records from above down: respiratory air flow, respiratory minute volume (Resp. V_m), blood flow and conductance in radial and femoral vascular beds, heart-rate and arterial blood pressure (B.P.). Stimulus marker shows period of injection. Between *A* and *B*, atropine methyl nitrate (20 μg/kg) was administered close arterially to the hind limbs, reducing vasodilator response in the hind limb muscles. Between *B* and *C*, atropine methyl nitrate (0·2 mg/kg) was administered systemically. Note the much reduced vasodilator responses in the muscles of both the fore- and hind limbs.

a pressor effect only was observed of up to 60 mmHg from the resting mean level, which was in the range 100–135 mmHg. Occasionally a pure depressor effect was observed but this did not amount to more than a 10 mmHg fall from the resting mean level.

Heart-rate responses were extremely variable both in magnitude and direction (see Figs. 1, 5 and 6). To some extent they were dependent on the resting heart-rate which ranged from 170 to 220 beats/min and was itself a partial reflexion of the level of anaesthesia (the heart-rate tended to increase as the animal changed from deep surgical anaesthesia to the lighter level preferred during the experimental period). It was a common but not consistent finding that stimulation of the right carotid body evoked tachycardia while stimulation of the left carotid body in the same animal elicited bradycardia. As the heart-rate increased during the course of the experiment, so tachycardia became the more common response from both sides and, when a stable level of anaesthesia had been attained with a hypersynchronous spindling e.e.g., tachycardia ranging from 3 to 20 beats/min usually occurred from 'resting' levels of 200–220 beats/min. The only exception to these generalizations was that the cynanide solutions invariably evoked a bradycardia, usually between 5 and 20 beats/min, from these same 'resting' levels.

The increase of vascular conductance in the muscles of the fore- and hind limbs began 12–15 sec after the start of the injection, lasted for 1½–2 min and regularly reached a peak of 2–3 times (Fig. 1) and occasionally 3–4 times above the resting levels. Smaller dilatations of 50–100 % were often seen, particularly at the beginning of the experimental period. The effect of the level of anaesthesia on these responses was tested in four experiments. All dilator responses were reduced, abolished or even converted to constrictor responses when the depth of anaesthesia was increased by administering Althesin, either as a bolus injection (0·5–1·0 mg/kg I.V.) or by increasing the infusion rate (Fig. 2.). Sometimes a second phase of dilatation began to develop 20–30 sec after the beginning of the first (see Fig. 4). This second phase which led to peak increases in conductance of 20–100 % from control levels outlasted the stimulus by up to 5 min.

In the mesenteric vascular bed there was a rapid decrease in conductance at the onset of chemoreceptor stimulation, usually by 12–15 % (85 % of experiments) but sometimes by as much as 40 % (15 % of experiments) (Fig. 2). This constriction, which usually resulted in a reduction in mesenteric flow, lasted 15–20 sec. It was sometimes followed by a rebound increase in flow and conductances as in Fig. 6 and sometimes by a smaller constriction which lasted 2–3 min (see Fig. 3). An increase in the depth of anaesthesia usually reduced the mesenteric vascular response (Fig. 2).

Renal vascular conductance frequently decreased by some 10–25 % (70 % of experiments) on chemoreceptor stimulation (Fig. 5) although there was often little change in renal blood flow due to the concomitant rise in blood pressure. However, when the effect of chemoreceptor stimulation was large as judged by the other autonomic responses (30 % of experiments) there was a rapid and powerful constriction in the renal vascular bed which lasted 15–20 sec, producing a 40–50 % reduction in renal conductance and resulting in a substantial reduction (35–40 %) in renal flow. This was often followed by a dilator component, when flow and conductance increased by 20–30 % above the control level before returning to the resting state.

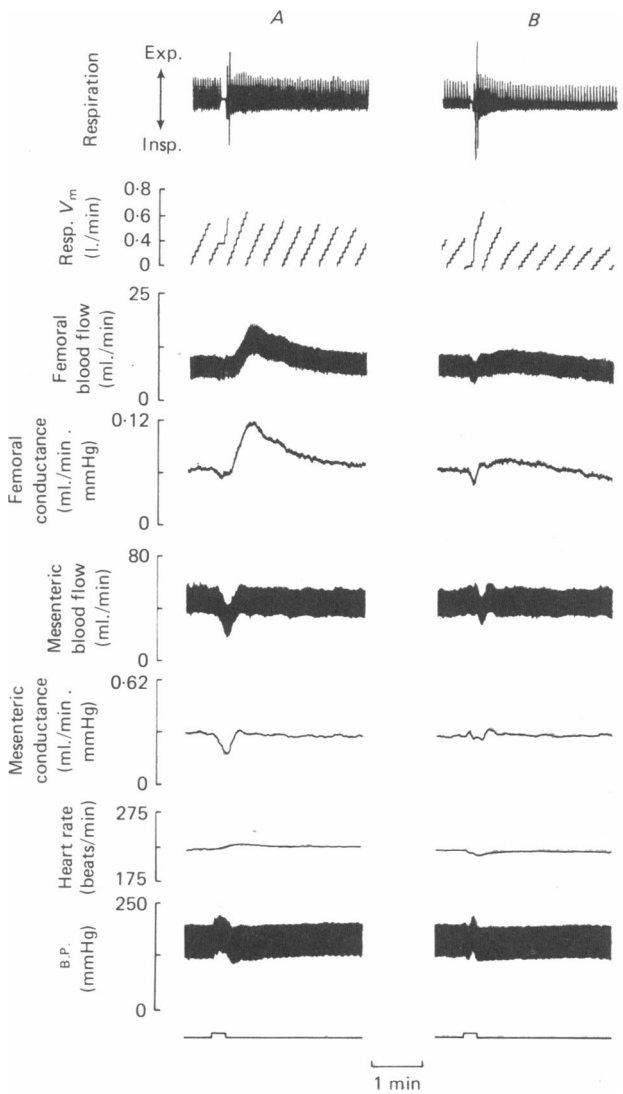

Fig. 2. Cat, Althesin. Effect of increased depth of Althesin anaesthesia on the response evoked on stimulation of carotid chemoreceptors with saline equilibrated with CO_2. Records from above down, respiratory air flow, respiratory volume (Resp. V_m), blood flow and conductance of femoral artery and cranial mesenteric artery vascular beds, heart rate, arterial blood pressure (B.P.) and stimulus marker. *A*, before and *B*, after a bolus injection of Althesin (2 mg/kg I.V.) in addition to the continuous infusion of Althesin (6 mg/kg I.V.).

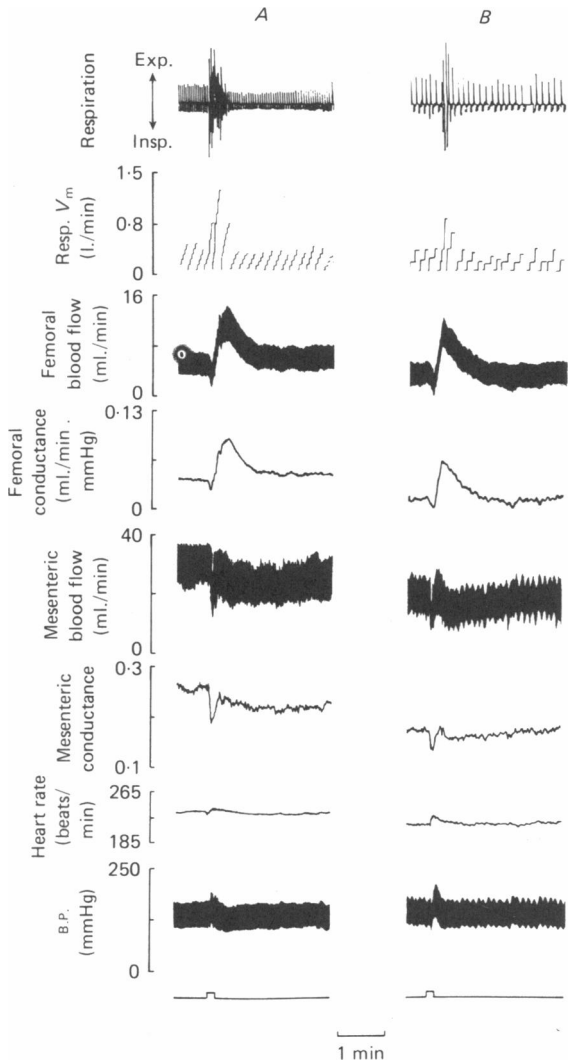

Fig. 3. Cat, Althesin. Effect of vagotomy on response evoked on stimulation of carotid chemoreceptors with inorganic phosphate solution. Records as in Fig. 2: *A*, before and *B*, after vagotomy.

Chemoreceptor stimulation also evoked an abrupt decrease in paw volume, followed by a return to the resting level over the next 2–3 min (Fig. 4): this indicates cutaneous vasoconstriction.

Similarity of the response to the alerting stage of the defence reaction

The response after vagotomy and muscular paralysis. These experiments were performed because of previous suggestions that tachycardia and peripheral vasodilatation in response to chemoreceptor stimulation are secondary to lung inflation. After vagotomy (performed in fifteen experiments), tachycardia ranging from 2 to

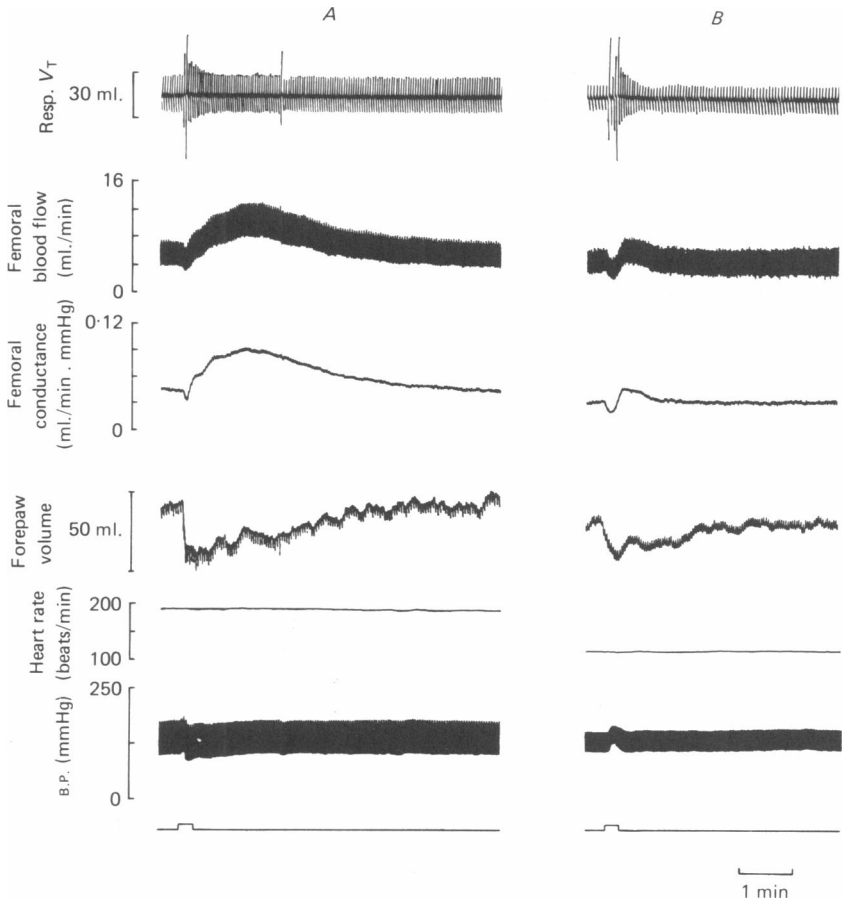

Fig. 4. Cat, Althesin. Effect of propranolol on responses evoked on stimulation of carotid chemoreceptors with saline equilibrated with CO_2. Records from above down: respiratory tidal volume (Resp. V_T), blood flow and conductance in femoral artery vascular bed, forepaw volume, heart rate arterial blood pressure (B.P.) and stimulus marker. *A*, before and *B*, after propranolol (0·3 mg/kg I.V.).

25 beats/min became the predominant cardiac response to chemoreceptor stimulation. A bradycardia of up to 10 beats/min occurred in some tests, but it was usually preceded by a slight tachycardia and invariably followed an initial pressor response and so was assumed to be of baroreceptor reflex origin. In all experiments the muscle vasodilator responses were at least as large after vagotomy as before (Fig. 3). Thus, these particular features of the response were not initiated by pulmonary vagal afferents. Vagotomy often reduced vascular conductance in the mesenteric bed and kidney, but chemoreceptor stimulation still evoked substantial vasoconstriction in both territories (see Fig. 3).

As the pattern of cardiovascular response was also essentially unchanged in artificially ventilated, paralysed animals (seven experiments), the tachycardia and muscle vasodilatation were not dependent on lung inflation. Moreover, the muscle

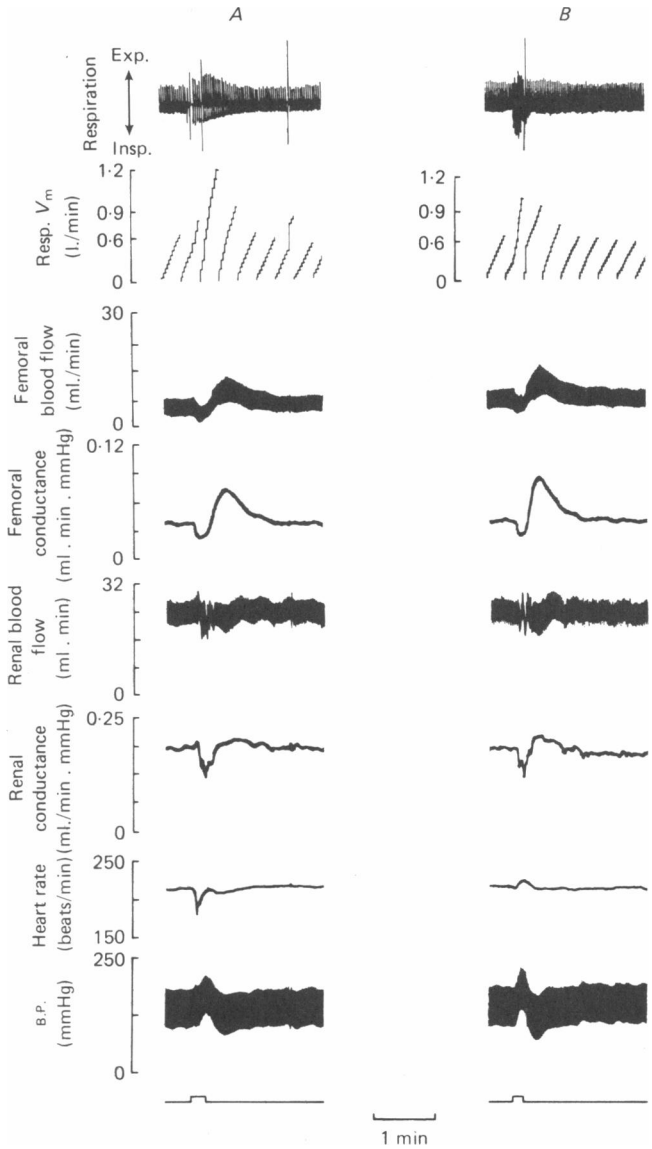

Fig. 5. Cat, Althesin. Comparison of responses evoked on carotid chemoreceptor stimulation and stimulation of radial nerve. Records as in Fig. 2, except blood flow and conductance in left renal artery vascular bed. *A*, chemoreceptor stimulation with saline equilibrated with CO_2, and *B*, radial nerve stimulation with pulses of 1·0 mA and 0·5 msec duration at 40 Hz for 10 sec.

dilatation was not a consequence of muscle contraction, as it was unaffected by muscular paralysis with gallamine.

The effects of pharmacological blocking agents on the muscle vasodilatation. The pattern of responses produced by chemoreceptor stimulation was so closely reminiscent of the alerting stage of the defence reaction that the muscle vasodilatation would be expected to be at least in part mediated by cholinergic dilator nerve fibres. In the

eight experiments in which it was administered, atropine methyl nitrate or atropine sulphate given close arterially (10–20 μg/kg) or systemically (0·1–0·2 mg/kg) substantially reduced or abolished muscle vasodilations of 100–150% (Fig. 1). Guanethidine (3 mg/kg I.V.) usually abolished the atropine-resistant dilatations or, when given as the first drug (four experiments), reduced dilatations of 100–150% by 50–60%. Thus a proportion of the muscle dilatation was due to inhibition of sympathetic vasoconstrictor tone. The late dilatation which often followed the initial response was abolished by propranolol (0·1–0·5 mg/kg I.V.) in the four experiments in which it was tested (Fig. 4) and thus was attributed to circulating adrenaline. It is concluded that the activation of cholinergic fibres, inhibition of constrictor fibres and release of adrenaline all contributed in various proportions in individual animals to produce muscle vasodilatation.

The resemblance of the response to that produced by stimulation of nociceptive afferents in peripheral nerves. In the conscious and high decerebrate cat stimulation of cutaneous afferents by pinching the skin of the paw or ear, or by application of electrical stimuli, evokes the behavioural and autonomic components of the defence reaction (Abrahams *et al.* 1960, 1964). In the cat anaesthetized with Althesin, electrical stimulation of the radial nerve or heavy pinching of the ear elicited a pattern of response which was almost indistinguishable from that evoked by chemoreceptor stimulation (Fig. 5). The only exceptions were that bradycardia never occurred with noxious stimuli and the effects on respiration were smaller. The full pattern of the response to noxious stimuli could also be obtained in vagotomized and curarized animals. Moreover, pharmacological blocking agents had the same effects in each individual animal on the muscle vasodilatation evoked by noxious stimuli as on that evoked by chemoreceptor stimulation.

The resemblance to the response to electrical stimulation in forebrain defence areas. In three experiments a monopolar steel electrode was positioned in the amygdaloid or hypothalamic defence areas or in the amygdalo-hypothalamic pathway and was used to evoke the autonomic components of the alerting stage of the defence reaction as described by Timms (1981). In each animal the pattern of response evoked was almost indistinguishable from that evoked by carotid chemoreceptor stimulation (Fig. 6). The main differences were in the extent of the hyperventilation which was usually more marked on chemoreceptor stimulation and in the heart-rate change which on defence area stimulation is typically a tachycardia with a bradycardia on the cessation of the stimulus (see Fig. 6).

Sham rage and the chemoreceptor response in the decerebrate animal. The high decerebrate preparations regularly showed bursts of sham rage characterized by hyperventilation, increase in arterial blood pressure, tachycardia except when the heart-rate was already greater than 350 beats/min, muscle vasodilatation of 100–200%, pupil dilatation, retraction of the nictitating membranes, pilo-erection and violent struggling and clawing movements. This pattern of response could be reproduced by stimulation of the peripheral chemoreceptors (Fig. 7). The cardiovascular and other autonomic responses could also be evoked in the paralysed and artificially ventilated preparation (three experiments) although the magnitude of individual components was somewhat reduced and the tendency towards 'spontaneous' sham rage was noticeably less. The muscle vasodilatation which occurred in the paralysed animal and which was therefore not secondary to muscle contraction was considerably

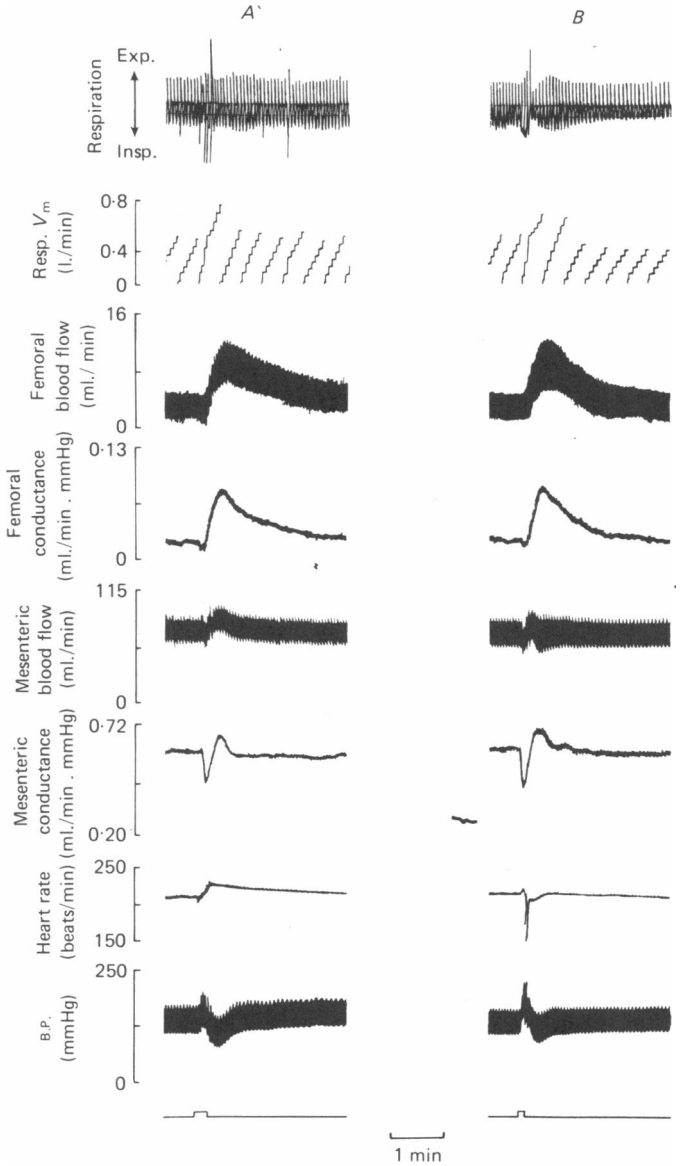

Fig. 6. Cat, Althesin. Comparison of responses evoked on carotid chemoreceptor stimulation and electrical stimulation in the ventral amygdalofugal pathway. Records as in Fig. 2. *A*, chemoreceptor stimulation with inorganic phosphate solution; *B*, stimulation in the ventral amygdalo-fugal pathway with pulses of 200 μA and 2 msec duration at 80 Hz for 10 sec.

reduced in all three experiments by atropine (0·2 mg/kg I.V.): dilatations of 100–500 % were reduced by at least 75 %. In one experiment a small dose of chloralose (20 mg/kg) was given to the otherwise unanaesthetized preparation. This substantially reduced the muscle dilatation of sham rage and that produced by chemoreceptor stimulation from 100 to 20 % (see Fig. 7).

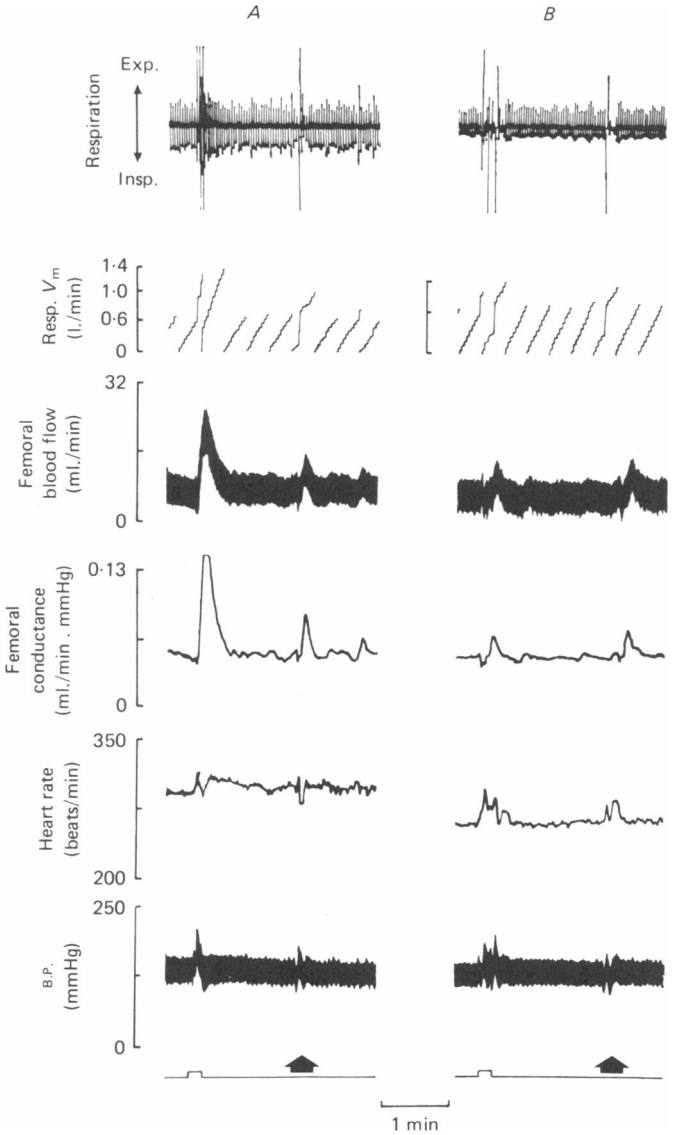

Fig. 7. High decerebrate cat. Effect of administration of chloralose on responses evoked on carotid chemoreceptor stimulation and during spontaneous sham rage. Records as in Fig. 1, except that flow and conductance recordings are from femoral artery only. Stimulus markers in *A* and *B* indicate injection of inorganic phosphate solution via the lingual artery, the arrows indicate occurrence of spontaneous sham rage. *A*, before and *B*, 10 min after, injection of α-chloralose (10 mg/kg I.V.).

DISCUSSION

This study has shown that activation of the carotid chemoreceptors by close arterial injection of a variety of stimuli in cats anaesthetized with Althesin causes, in addition to hyperventilation, a complex pattern of cardiovascular response. The changes in the peripheral circulation include dilatation of resistance vessels in skeletal muscle (preceded by a small constriction) and vasoconstriction in mesenteric, renal and cutaneous vasculature. There is no indication that the injections caused significant, direct stimulation of the carotid baroreceptors in view of the widespread peripheral vasoconstriction, even initially in skeletal muscle. Moreover, the muscle vasodilatation occurred whether or not the arterial blood pressure rose and even during bilateral carotid occlusion (Marshall, 1981), so this component of the response is also elicited independently of baroreceptor stimulation.

The heart-rate fell in some experiments, and rose in others, but when tachycardia occurred neither this change nor the muscle vasodilatation could be explained as secondary effects of lung stretch afferent stimulation during hyperventilation, as they have been in the dog (Daly & Scott, 1962). In the present experiments, the magnitudes of the vasodilatation and tachycardia were essentially the same no matter whether the animal was respiring spontaneously or under constant artificial ventilation, so any such secondary effects can hardly have been of significance. Indeed, MacLeod & Scott (1964) made a similar observation concerning secondary effects on heart-rate in their experiments on cats anaesthetized with a mixture of chloralose and urethane. The present results support their suggestion that the apparent disparity between the species in part reflects the particularly strong ventilatory response of the dog to carotid chemoreceptor stimulation. We conclude therefore that the pattern of response seen in the cat anaesthetized with Althesin on stimulation of the carotid chemoreceptors can be attributed directly to the increase in chemoreceptor activity.

There is evidence both from previous investigations (Bizzi *et al.* 1969; Hilton & Joels, 1965) and from the present experiments on high decerebrate cats that in the absence of anaesthesia, albeit after removal of descending inhibitory inputs, chemoreceptor stimulation can provide an excitatory input to the brain stem defence areas. This is in accord with previous observations that units within the hypothalamic defence area defined by Abrahams *et al.* (1960) respond to hypoxia and hypercapnia (Cross & Silver, 1963) and to electrical stimulation of the sinus nerve with stimulus parameters chosen specifically to stimulate chemoreceptor afferent fibres (Thomas & Calaresu, 1972).

The resemblance between the pattern of response we have demonstrated on chemoreceptor stimulation and that evoked by direct stimulation of the brain stem defence areas is striking: the muscle vasodilatation evoked on chemoreceptor stimulation was usually abolished by atropine, indicating that it was mediated by cholinergic nerve fibres, and it was accompanied by pupillary dilatation, retraction of the nictitating membrane and pilo-erection, all of which are characteristic features of the alerting stage of the defence reaction in the cat (Abrahams *et al.* 1960, 1964). Thus, the present experiments provide further evidence that chemoreceptor stimulation can activate the hypothalamic and brain stem defence areas. This view

is strengthened by our observation that in the cat anaesthetized with Althesin the response evoked by nociceptive stimuli, which are known to activate the defence areas in the conscious animal, was almost indistinguishable from that evoked by chemoreceptor stimulation. The finding that the muscle vasodilatation evoked by chemoreceptor stimulation and by nociceptive stimulation was not always abolished by atropine and was then sensitive to guanethidine is consistent with this view for, while the muscle vasodilatation evoked by stimulation in the defence areas of the hypothalamus and mid-brain is mediated predominantly by cholinergic vasodilator fibres, that elicited by stimulation in the defence area of the medulla is due to inhibition of sympathetic vasoconstrictor tone (Coote, Hilton & Zbrozyna, 1973). It was suggested by Coote *et al.* (1973) that natural stimuli would simultaneously excite all the defence areas situated along the length of the brainstem, and our findings now provide evidence for this idea. As electrical stimulation in the defence areas (Grant, Lindgren, Rosén & Uvnäs, 1958) and hypoxic stimulation of the carotid bodies (Critchley, Ungar & Welburn, 1973) have both been shown to cause liberation of adrenaline from the adrenal medulla, the second phase of dilatation which appeared in some experiments, and which was blocked by the β-antagonist, propranolol, is easily explained.

Whereas direct stimulation of the defence areas always evokes tachycardia, we found that it only became the predominant response to chemoreceptor stimulation after vagotomy or at lighter levels of anaesthesia. In previous studies, all of them on cats anaesthetized with chloralose, chloralose and urethane, or pentobarbitone, chemoreceptor stimulation has produced a variety of different effects on heart-rate. Some have reported a primary bradycardia, (e.g. MacLeod & Scott, 1964) in part due to excitation of the vagus, in part to inhibition of sympathetic fibres. On the other hand Little & Öberg (1975) found that vagotomized cats under constant artificial ventilation showed a tachycardia. Direct recordings of cardiac sympathetic activity (Trzebski, Lipski, Majcherczyk, Szulczyk & Chruscielewski, 1975; Montarolo, Passatore & Raschi, 1976) have shown that this output is engaged by chemoreceptor stimulation under these conditions. In addition, under the very same experimental conditions, it is known that the cat shows little or no vagal tone, but some activation of presumed cardio-inhibitory fibres by carotid chemoreceptor stimulation can be demonstrated (Kunze, 1972; McAllen & Spyer, 1978*a*). Furthermore, the effect of enhanced central inspiratory drive is to increase sympathetic activity (Preiss, Kirchner & Polosa, 1975; Lipski, Coote & Trzebski, 1977) and inhibit (or disfacilitate) cardio-inhibitory vagal neurones (Katona, Lipson & Dauchot, 1977; McAllen & Spyer, 1978*a*, *b*). Thus, on the basis of these results, the final outcome as far as the heart is concerned is ambiguous and bound to depend on a variety of circumstances. It seems likely that, at lighter levels of anaesthesia with Althesin which are the very conditions favouring the development of the active muscle vasodilatation, the tachycardia of the alerting response becomes superimposed and predominates over all other cardiac effects which chemoreceptor stimulation may induce.

As mentioned in the Introduction the more conventional anaesthetics, like chloralose and barbiturate, prevent normal activation of the defence areas by afferent pathways from the amygdala and peripheral nociceptive inputs. Chloralose, in particular, distorts the response in that it allows the rise in arterial pressure and the

tachycardia to be expressed but suppresses the muscle vasodilatation (Abrahams *et al.* 1960; Hilton & Zbrożyna, 1963). We observed a similar differential effect on the cardiovascular changes accompanying sham rage elicited by chemoreceptor stimulation when a small dose of chloralose was administered to a high decerebrate preparation. By contrast, cats fully anaesthetized with Althesin show all the autonomic components of the alerting stage of the defence reaction on stimulation in the amygdaloid defence area (Timms, 1976, 1981) and, as we have shown, when exposed to a noxious cutaneous stimulus. All this evidence leads to the conclusion that activation of the hypothalamic and brain stem defence areas is an integral part of the response to carotid chemoreceptor stimulation, which cannot be seen in animals anaesthetized with the more conventional anaesthetics. There is the interesting possibility that some of the discrepancies in the literature concerning the heart-rate responses to chemoreceptor stimulation may be explained by the distorting actions peculiar to chloralose on transmission in the central nervous system.

It has been reported recently that when a small, additional dose of Althesin is administered to a cat already adequately anaesthetized with this agent, the autonomic response evoked by stimulation in the amygdaloid defence area is unchanged (Timms, 1981). By contrast, we found this procedure to change the response to chemoreceptor stimulation to one of bradycardia and a predominant peripheral vasoconstriction, even in skeletal muscle. This indicates a certain lack of security of conduction in the chemoreceptor pathway from the medulla to the defence areas, and points to the possibility that, in the conscious animal also, without any anaesthetic, the ability of a chemoreceptor stimulus to activate the defence areas might depend on the strength of the stimulus and the existing level of activity in the defence areas. Thus, a chemoreceptor stimulus of low potency to the defence areas could evoke the 'primary' chemoreceptor response, perhaps complicated by secondary effects due to hyperventilation, whilst, on stronger stimulation, the autonomic components of the alerting stage of the defence reaction would be fully expressed.

From our conclusions so far chemoreceptor stimulation in the conscious animal might be expected to produce the behavioural and autonomic signs of alerting, although not necessarily leading to a full defence reaction. Rutherford & Vatner (1978), who stimulated the carotid chemoreceptors with NaCN in conscious dogs, made no comment on any behavioural changes, but the pattern of cardiovascular response was like that of the alerting response, comprising tachycardia, vasoconstriction in renal and mesenteric vasculature and vasodilatation (preceded by constriction) in the hind limbs. The authors attributed the tachycardia and muscle vasodilatation to reflexes resulting from hyperventilation for, when the animal was paralysed and artificially ventilated or vagotomized, chemoreceptor stimulation evoked constriction in muscle and bradycardia. Whilst it might be expected that secondary reflexes induced by hyperventilation would be strong in the dog their conclusion would need to be tested in other experimental conditions for the interventions must have been disturbing and they did cause substantial changes in the base line state of the cardiovascular system.

In their extensive studies on the effects of hypoxia in rabbits, Korner and his co-workers (see Korner, 1971, for refs.) have argued that the cardiovascular response is due predominantly to peripheral chemoreceptor stimulation rather than to the

central or local effects of low P_{O_2}, and that the reflex effects induced by hyperventilation on heart-rate or peripheral vasculature during severe hypoxia are not profound (Korner, 1965). When conscious rabbits were exposed to hypoxia they displayed clear signs of behavioural alerting, sniffing in the corners of the experimental chamber 'as though looking for a means of escape' (Korner, Uther & White, 1969). It has recently been shown in our laboratory that this behaviour is also evoked in the conscious rabbit by electrical stimulation in the hypothalamic defence area (Azevedo, 1981). In Korner's experiments the heart-rate fell – a not unexpected effect of chemoreceptor stimulation, see above – but the pattern of response in the peripheral vasculature was similar to that of the alerting response with vasoconstriction in the splanchnic area and kidney and dilatation in skeletal muscle (Uther, Hunyor, Shaw & Korner, 1970). The muscle vasodilatation was ascribed to the effects of circulating adrenaline, as it was replaced by vasoconstriction after adrenalectomy or propranolol administration. But both adrenalectomy and propranolol themselves led to a large vasodilatation in the muscle, and in recent experiments on the muscle vasodilatation evoked by electrical stimulation in the defence areas of the rabbit it was not possible to ascertain the mechanism responsible, one of the problems being that the pharmacological blocking agents used themselves reduced the vascular resistance in the muscle vasculature (Azevedo, 1981). Thus, from experiments performed so far, stimulation of peripheral chemoreceptors in the conscious animal may produce the autonomic as well as the behavioural features of the alerting response. Further experiments will be necessary to be sure of the extent to which this generalization holds good.

In conclusion, the present experiments have demonstrated that stimulation of the carotid chemoreceptors in cats anaesthetized with Althesin evokes a pattern of cardiovascular response which comprises the characteristic features of the alerting stage of the defence reaction superimposed upon the cardiovascular changes which in previous studies on anaesthetized animals have been designated the primary and secondary responses to chemoreceptor stimulation. The evidence suggests that Althesin allows a more complex expression of the response than the more conventional anaesthetics like chloralose and barbiturates because it does not have the same depressant action on synaptic transmission through the brain stem defence areas. It has been inferred previously (Korner *et al.* 1969; Korner, 1971), from experiments involving removal of large sections of the brain, that suprabulbar regions play a role in integrating the response to chemoreceptor stimulation in the conscious animal. The present experiments indicate that at least part of this integration is carried out by the defence areas – those regions of the brain which are already known to integrate the autonomic and behavioural responses to alerting stimuli. The implications of this are that strong chemoreceptor stimulation in the conscious animal may, by itself or in combination with other alerting stimuli, elicit the full pattern of the alerting response.

Perhaps of greater physiological significance is the possibility that the input from peripheral chemoreceptors may play a hitherto unforseen role in setting the level of arterial blood pressure (Guertzenstein, Hilton, Marshall & Timms, 1978; Hilton 1980) and that this input may interact with other incoming afferent information in a hitherto unexpected manner which has important consequences for cardiovascular

control. The latter proposal has already been substantiated by the demonstration that, just as electrical stimulation in the defence areas may suppress the baroreceptor reflex (Coote, Hilton & Perez-Gonzalez, 1979), so may activation of peripheral chemoreceptors (Marshall, 1981).

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