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VENTRAL MEDULLARY RELAY NEURONES IN THE PATHWAY FROM THE DEFENCE AREAS OF THE CAT AND THEIR EFFECT ON BLOOD PRESSURE

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SUMMARY

1. In cats anaesthetized with Althesin, the efferent descending pathway from the brain-stem defence areas has been traced through the medulla by identifying sites at which electrical stimulation evoked the characteristic pattern of the visceral alerting (defence) response. This response includes an increase in arterial blood pressure resulting from increased heart rate and cardiac output and vasoconstriction in renal and splanchnic beds, accompanied by active vasodilatation in skeletal muscle.

2. The efferent pathway runs as a narrow strip, about 3 mm from the mid line, ventral to the superior olive and the nucleus of the trapezoid body, extending caudally to the rostral portion of the inferior olive where it lies ventral to the facial nucleus. It was found to lie very close to the ventral medullary surface just rostral to and within the area at which bilateral topical application of glycine results in a profound fall in arterial blood pressure and cessation of respiration.

3. On bilaterial application of glycine to the sensitive area of the ventral medulla, the visceral alerting response evoked by stimulation in the defence areas of the amygdalo–hypothalamic complex, or the mid-brain central grey or tegmentum, was attenuated in parallel with the fall in arterial pressure, the vasoconstrictor responses being most strongly reduced. As soon as arterial blood pressure had fallen to its lowest level the visceral alerting response was virtually abolished.

4. A small radio-frequency lesion made in the ventral medullary efferent pathway, in the rostral part of the 'glycine-sensitive area', had the same effect as that produced by unilateral application of glycine: it resulted in little respiratory or cardiovascular effect itself, but application of glycine to the contralateral area then produced the full effect otherwise seen only on bilateral application of glycine.

5. It is suggested (1) that the effects of glycine result from blockade of a synaptic relay, close to the ventral surface of the medulla, in the efferent pathway from the defence areas to the preganglionic sympathetic neurones, and (2) that the neurones which receive an input from the alerting (defence) areas normally provide an essential, tonic excitatory drive to the sympathetic output and probably to respiration also. After sudden withdrawal of this drive, vasomotor tone and the normal level of arterial blood pressure are not maintained.

INTRODUCTION

The growing interest in the chemical sensitivity of the ventral surface of the medulla oblongata has led to claims of important roles for this region in cardiovascular and respiratory control (for references see Feldberg, 1981; Schlaefke, 1981). For instance, the topical application of a number of drugs to the ventral medulla can cause profound changes in arterial blood pressure. Feldberg & Guertzenstein (1972) first showed that the large fall in arterial pressure evoked by an intraventricular injection of sodium pentobarbitone could be reproduced if the drug was applied to a restricted area of the ventral medulla via Perspex cups placed bilaterally just caudal to the trapezoid bodies. Later, Guertzenstein (1973) demonstrated a similar effect when glycine was applied bilaterally to the same region. Guertzenstein & Silver (1974) localized the glycine-sensitive area more precisely and showed that bilateral lesions at this site, or a unilateral lesion combined with application of glycine to the other side of the medulla, produced the same profound fall in arterial pressure.

It had already been proposed by Feldberg & Guertzenstein (1972) that a group of superficial medullary neurones must play a fundamental role in the maintenance of arterial pressure. Since glycine acts preferentially on cell bodies, producing inhibition by hyperpolarization (Werman, Davidoff & Aprison, 1968), the results of Guertzenstein & Silver (1974) lent weight to that hypothesis; but they did not relate their findings to any particular neuronal system involved in cardiovascular regulation. The present experiments were carried out because of the likelihood that the efferent pathway from the defence areas of the hypothalamus and brain stem runs through the glycine-sensitive area. This is the pathway by which the cardiovascular and other visceral components of the alerting reaction are evoked. Its exact course has not been previously described in detail, though it is known that it takes a ventral route through the mid-brain and medulla (Lindgren & Uvnäs, 1953; Lindgren, Rosén, Strandberg & Uvnäs, 1956; Abrahams, Hilton & Zbrozyna, 1960). Furthermore, the results of Schramm & Bignall (1971) indicate that the pathway for active muscle vasodilatation, which is a characteristic feature of the pattern of cardiovascular response, lies in a very superficial position at the level of the glycine-sensitive area. We have therefore investigated the possibility that the fall in arterial pressure described by Guertzenstein & Silver (1974) is due to destruction or blockade of the efferent pathway from the defence areas.

In the present study we have traced the efferent pathway for the visceral alerting (defence) response through the ventral medulla. We have also investigated the effect of glycine applied to the ventral medulla and of lesions in the pathway on the patterned response to defence area stimulation, as well as on the resting state of the cardiovascular and respiratory systems. Our results lead us to suggest that glycine, applied in this way, exerts its effect by blocking relays in the descending pathway from the defence areas, and hence that the neurones in the glycine-sensitive area which relay the visceral alerting response are of particular importance for maintaining total peripheral resistance. These neurones could comprise a functional nucleus of great physiological significance, in that their activity may be essential for setting the general level of arterial blood pressure in the awake or anaesthetized organism.

Preliminary accounts of some of these experiments have already been published (Guertzenstein, Hilton, Marshall & Timms, 1978; Hilton, Marshall & Timms, 1980).

METHODS

Experiments were performed on twenty-six cats in which anaesthesia was induced by brief exposure to a mixture of oxygen, halothane and nitrous oxide followed by a single injection of alphaxalone–alphadolone (Althesin, Glaxo; 4–5 mg total steroids/kg, I.V.). Anaesthesia was maintained with a continuous intravenous infusion of Althesin as described by Timms (1976, 1981). The rate of infusion was 8–16 mg/kg . h during surgery and 3–6 mg/kg . h in the experimental period. Depth of anaesthesia was carefully monitored with the aid of a fronto-occipital e.e.g., recorded via two stainless-steel screws (10 B.A.) set in the cranium, and displayed on a storage oscilloscope (Telequipment DM64). Rectal temperature was maintained at 37–38 °C using a servo-controlled blanket device.

Arterial blood pressure was recorded from a common carotid or femoral artery via a pressure transducer (Bell & Howell) and heart rate was derived from the pressure pulse by an instantaneous rate-meter (Miller, 1976). Blood flow was recorded in each of two arteries: either in each femoral artery, or in one femoral or brachial artery and in one of the following: the left renal or cranial mesenteric arteries, or (in a few experiments) in the ascending aorta. The equipment used consisted of cuff-type transducers (Biotronex Laboratory, Micron Instruments or S.E. Labs) fitted to electromagnetic flow amplifiers (S.E. Medic, S.E. Labs). These were later calibrated *in vitro* using constant flow perfusion of a length of freshly excised artery. Flow transducers were mounted as follows: on (a) the femoral artery from a medial approach, distal to the deep femoral branch with the inguinal fat pad isolated and divided, and on (b) the brachial artery also from a medial approach; in each case the circulation to the paw was excluded by a stout ligature around the ankle or wrist (since the aim was to record mainly limb muscle flow); on (c) the renal artery using a retroperitoneal approach; on (d) the cranial mesenteric artery intraperitoneally, and on (e) the ascending aorta using an approach from the left side, the thorax then being resealed and negative pressure re-established so that spontaneous respiration could be resumed. Zero flow in peripheral arteries was obtained periodically by means of an occluding snare placed distal to the flow transducer. Zero flow in the aorta was estimated from the flow wave form. Regional vascular conductance or total peripheral conductance (a beat-by-beat division of arterial or aortic flow by arterial pressure) was computed on-line by custom-built electronic dividers. In some experiments, changes in the mesenteric vascular bed were investigated by monitoring changes in perfusion pressure (using a Bell & Howell transducer) during constant flow perfusion by means of a roller-pump (Watson Marlow Ltd.; type MHRE). In these experiments, the animal was given heparin (1000 i.u./kg, I.V.) and prednisolone sodium phosphate (Codelsol; Merck, Sharp & Dohme Ltd.; 2 mg/kg subcutaneously). The cranial mesenteric artery was then cannulated using an approach from the right side of the abdomen and perfused with blood taken from the femoral artery. The pump was set to deliver at a flow rate similar to the resting flow in the perfused artery just prior to its cannulation, as determined using an electromagnetic flowmeter. Respiration was recorded as tracheal air-flow by means of pneumotachograph (Metabo, type 000) and as expiratory minute volume by integration of the air-flow record (Devices M19 conditioning units).

The ventral surface of the medulla was exposed as described by Guertzenstein (1973), the dura being left intact until immediately before medullary stimulation was begun or glycine applied. When glycine was used it was applied to the medullary surface via oval Perspex 'cups' similar to those illustrated by Guertzenstein (1973). Glycine was dissolved routinely in normal saline at a concentration of 100 or 200 mg/ml, 10 μl of one of these solutions at 37 °C being placed inside each cup as described by Guertzenstein (1973). As far as could be judged, the higher concentration was slightly more effective than the lower. Sodium chloride solutions of the same tonicity or acidified to the same pH as the glycine solutions caused no measurable cardiovascular or respiratory effects when applied via the cups and restricted to the 'glycine-sensitive area' (cf. Guertzenstein & Silver, 1974).

In three experiments we tested the effect of glycine applied in this way on the baroreceptor reflex. A blind-sac preparation was made of the carotid sinus region on one side, and, to evoke a reflex response, the sac was inflated using oxygenated, normal saline to a static pressure of 200–220 mmHg via a cannula in the external carotid artery, with the common carotid artery temporarily occluded. Sinus pressure was monitored from a side-arm of the cannula using a transducer (Bell & Howell) and displayed, with all other recorded variables, on pen recorders (Devices).

With the animal in a stereotactic frame, stimulating electrodes were placed in the defence areas of the hypothalamus or mid-brain or in the ventral amygdalofugal pathway via small, dorsal

craniotomies. The areas were identified on the basis of their response to electrical stimulation (see Timms, 1981) and the electrodes fixed firmly in position using dental cement. Sites in the ventro-lateral medulla were likewise reached using a dorsal approach and the more medial sites via the ventral approach described above. The monopolar, stainless-steel electrodes were made from surgical needles (Holborn Instrument Co. size 2 or 5) which had been sharpened electrolytically and coated with Araldite 985 (Ciba-Geigy) except for the tip. The length of the bare tip of electrodes used to stimulate in the ventral amygdalofugal pathway was 0·07–0·15 mm, and in the hypothalamus, mid-brain or medulla 0·03–0·05 mm. A Digitimer was used to drive a stimulus isolation unit (Devices) and constant current unit (Grass, CCUIA) via a gated pulse generator (Devices). Trains of rectangular, cathodal pulses, of 2 ms duration at 80 Hz were delivered for 5–15 s. Peak-to-peak current was measured throughout the stimulation period as the voltage drop across 1 kΩ on the indifferent side and displayed on a storage oscilloscope (Telequipment, DM53A). The current used was: 100–250 μA in the ventral amygdalofugal pathway, 100–200 μA in the hypothalamus or mid-brain and 80–130 μA in the medulla.

Effective stimulation sites were marked with Prussian Blue spots made by the action of ferrocyanide on small deposits of iron made by passing 10–20 μA d.c. for 10–15 s with the electrode as anode (Green, 1958). In four experiments, a lesion was made within the ventral part of the medulla via the stimulating electrode using a radio-frequency device similar to that described by Aronow (1960). In these experiments, a stimulating electrode was used which had a bare tip of 0·05–0·1 mm. At the end of each experiment, the brain was fixed by perfusion and immersion in 10% formol–saline containing 1% potassium ferrocyanide. 50 μm sections were cut on the freezing microtome and stained with Neutral Red so that the locations of stimulation sites and lesions could be assessed microscopically.

RESULTS

Effect of bilateral application of glycine on cardiovascular and respiratory variables

Our initial experiments confirmed that bilateral application of glycine to a localized region of the ventral surface of medulla produces a profound fall in arterial blood pressure (Guertzenstein & Silver, 1974); we found that it also stopped all movements of respiration.

Unilateral application of glycine produced little or no change in arterial pressure (cf. Guertzenstein & Silver, 1974) or in respiration, even when the drug remained in contact with the medulla for up to 2 min. However, the subsequent application of glycine to the other side of the medulla, so that the sensitive region on each side was exposed to the drug, induced pronounced cardiovascular and respiratory changes which began within 15 s. Respiration became shallower and slower, and all respiratory movements ceased within 1·5–2 min. No matter whether artificial ventilation was begun before the glycine was applied or after breathing had stopped, the cardiovascular changes were the same: the main features of these changes are illustrated in Fig. 1. Mean arterial pressure fell by 20–60% from the control level of 105–135 mmHg, to reach a stable level of 50–90 mmHg within 1·5–5 min. The resting level of heart rate was 170–250 beats/min and this was always reduced, usually by less than 10% and never by more than 20%. Cardiac output, measured as flow in the ascending aorta, fell by 10–45% whilst total peripheral conductance increased by 20–50%. Vascular conductance in the femoral and renal beds either did not change or showed a slight increase of less than 15%. Our recordings from the mesenteric artery, using an electromagnetic flow transducer, indicated that a considerable increase in mesenteric conductance occurred at the same time as the fall in arterial pressure. However, the size of this change might not have been accurately registered by the flowmeter if

contact had been lost between the electrodes and the arterial wall as the perfusion pressure fell to low levels. Since the increase in total peripheral conductance seemed to have resulted largely from a vasodilatation in the splanchnic region, we thought it important to verify this result. We therefore carried out some experments in which the mesenteric artery was perfused at constant flow and the perfusion pressure monitored. These experiments confirmed that bilateral application of glycine to the ventral medulla induced substantial vasodilatation in the splanchnic region: there was a 45–70% increase in mesenteric conductance which occurred at the same time as a 50–60% fall in systemic pressure. In none of our experiments did the bilateral application of glycine induce a noticeable change either in the diameter of the pupils or in the position of the nictitating membrane.

The area of the ventral medulla to which glycine had to be applied bilaterally to produce the full pattern of change described is shown on the right-hand side of the diagram of the ventral medulla in Fig. 4*A*.

Effect of bilateral application of glycine on the visceral alerting (defence) and baroreceptor reflex responses

In each experiment, an electrode was implanted in one of the defence areas of the brain at a site at which electrical stimulation evoked the visceral components of the alerting stage of the defence reaction (the visceral alerting response). The full response pattern comprised vasoconstriction in the kidney and splanchnic area but vasodilatation in the hind limb, such that total peripheral conductance first fell then rose. There was a tachycardia, (often followed at the end of the stimulation period by a bradycardia), increased cardiac output and a rise followed by a fall in mean arterial pressure, together with hyperventilation, as shown in Figs. 1, 2 and 5. These changes were accompanied by dilatation of the pupils, retraction of the nictitating membranes and some piloerection at the base of the tail.

The actual sites of stimulation lay in the defence areas of the hypothalamus, mid-brain tegmentum or central grey matter, or in the ventral amygdalofugal pathway (Abrahams *et al.* 1960; Hilton & Zbrozyna, 1963; Timms, 1981). In the present experiments, the test response showed little or no attenuation even when the standard stimulus was repeated eight to twelve times (cf. Abrahams *et al.* 1960). However, after bilateral application of glycine to the sensitive area, and as soon as mean arterial pressure had fallen to 80–100 mmHg and respiration had ceased, the cardiovascular response to defence area stimulation was already substantially reduced, as shown in Fig. 1, and the stimulus evoked no respiratory movements. The vasoconstrictor components of the response were particularly strongly affected, as shown by the fact that the initial reduction in total peripheral conductance and the associated rise in arterial pressure were virtually abolished (Fig. 1*C*) and vasoconstriction was no longer elicited in renal or mesenteric beds. The increases in cardiac output, hind-limb conductance and heart rate were substantially reduced (Fig. 1*C*) as were the evoked pupillary dilatation and retraction of the nictitating membranes. By the time arterial pressure had stabilized at its lowest level (50–70 mmHg) defence area stimulation produced little effect apart from a reduced tachycardia, which persisted even after vagotomy, and sometimes a slight dilatation of the pupils. It seems unlikely that this depression of the alerting response was due to reduced

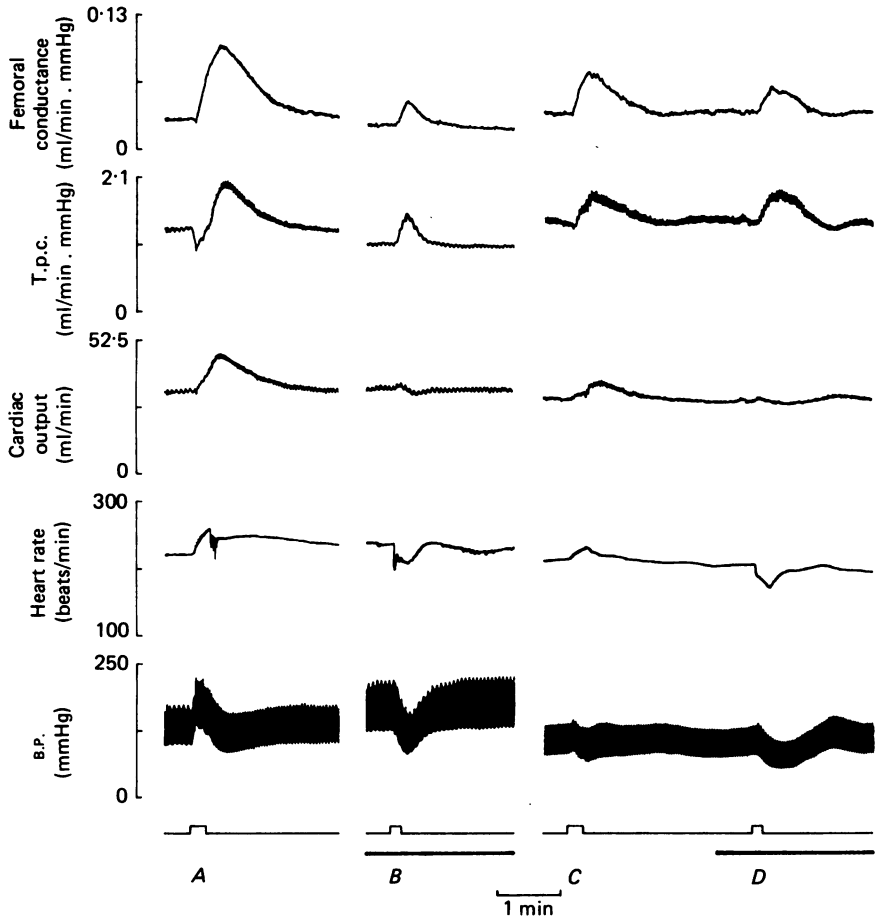

Fig. 1. Effect of the bilateral application of glycine to the 'glycine-senstive area' of the ventral medulla on resting cardiovascular variables and on the visceral alerting response and baroreceptor reflex response. Records, from below upwards, of arterial blood pressure (B.P.), heart rate, cardiac output (mean blood flow in the ascending aorta), total peripheral conductance (t.p.c.) and right femoral vascular conductance. Stimulus markers indicate: *A* and *C*, electrical stimulation at the same site in the right ventral amygdalofugal pathway for 15 s at 200 μA; *B* and *D*, increase of pressure to 200 mmHg for 10 s within the right carotid sinus blind-sac. Heavy lines below the stimulus markers indicate periods of bilateral common carotid artery occlusion. Between *B* and *C*, artificial ventilation was commenced and 10 μl glycine solution (100 mg/ml) was applied to the sensitive area on each side of the ventral medulla. Stimulus *C* was delivered about 7 min after the application of glycine.

perfusion of the brain and spinal cord, for even when arterial pressure had fallen to the lowest level induced by glycine, electrical stimulation in the dorsal medullary reticular formation still evoked a pronounced pressor response with vasoconstriction in all peripheral vascular beds.

After the bilateral application of glycine, the efficacy of the baroreceptor reflex, as judged by the cardiovascular response to inflation of a blind-sac preparation of the carotid sinus region, was dependent on the prevailing level of arterial pressure. A

significant change in arterial blood pressure and vascular conductance could seldom be evoked reflexly when mean pressure had fallen below 60–70 mmHg. At a time when arterial blood pressure had only fallen partway to the minimum level, and the response to defence area stimulation was already reduced, inflation of the carotid blind sac still elicited increases in regional and total peripheral conductance and a bradycardia, but now leading to a reduction in arterial pressure to about this minimum level (Fig. 1). In each experiment the reflex response was somewhat prolonged after glycine (Fig. 1). These results do not require that glycine-sensitive neurones are essential to the baroreceptor reflex pathway, as has been suggested by Wennergren & Öberg (1980) and McAllen, Neil & Loewy (1982).

Before the application of glycine, bilateral common carotid occlusion led to a vasoconstriction in the peripheral vascular beds and a rise in arterial pressure. After bilateral application of glycine, and when arterial pressure had fallen, the response to carotid occlusion was no longer obtainable: this is shown in Fig. 1. At least part of the explanation for this finding may be that at reduced levels of arterial pressure there is less baroreceptor activity to be affected by carotid occlusion.

The efferent pathway for the visceral alerting response in the ventral medulla

The full visceral alerting response could be elicited by electrical stimulation on, or close to, the surface of the ventral medulla. The sites from which this pattern of response was evoked lay in a narrow strip running longitudinally about 3 mm from the mid line on either side. Fig. 4, which shows the location of the histologically verified stimulation sites, was compiled from the twenty-one experiments of this type and consequently shows a scatter of positive sites wider than that seen in any single experiment. In each experiment, the pattern of cardiovascular response was the same as that evoked by stimulation in the fore- or mid-brain defence areas (see Fig. 2) with the exception that medullary stimulation evoked a hind-limb vasodilatation which had a slightly shorter latency to onset (1–3 s as against 3–5 s on defence area stimulation) and was sometimes larger in the ipsilateral limb.

The hind-limb vasodilatation evoked from the ventral medulla as part of the visceral alerting response was reduced by around 50% by atropine sulphate (0·2–0·3 mg/kg, I.V.), indicating that it was mediated, at least in part, by sympathetic cholinergic vasodilator neurones, as is the case for the alerting pattern of response evoked from the fore- and mid-brain defence areas (Abrahams *et al.* 1960; Hilton & Zbrozyna, 1963; Schramm & Bignall, 1971; Timms, 1981). Dilatation of the pupils occurred sometimes bilaterally, but sometimes only ipsi- or contralateral to the site of the medullary stimulation, whereas it always occurred in both eyes on defence area stimulation.

Examination of the histological sections of the brain prepared after each experiment revealed that the most effective stimulation sites lay within 500 μm, and often less than 200 μm, deep to the ventral surface of the medulla, or to the dorsal border of the pyramidal tract (at the medial extent of the band), as shown in Fig. 4*B*. The sites were most superficial within or just rostral to the glycine-sensitive area, as defined by Guertzenstein & Silver (1974). Stimulation at most sites within the glycine-sensitive area or caudal to the area itself evoked a pattern of response similar to the alerting pattern except that arterial pressure and heart rate fell. Stimulation even more

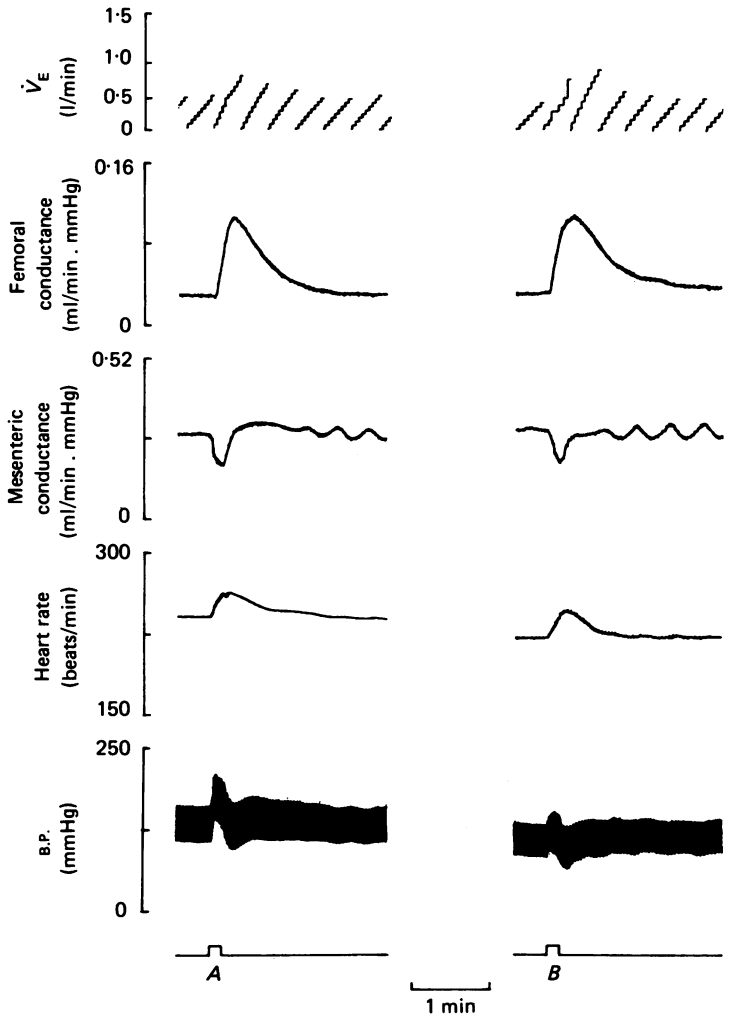

Fig. 2. Comparison of responses to stimulation in the forebrain defence areas and in their efferent pathway in the ventral medulla. Records, from below upwards, of arterial blood pressure (B.P.), heart rate, vascular conductance from cranial mesenteric and left femoral arteries, and respiratory minute volume ($\dot{V}_E$). Stimulus markers indicate: *A*, stimulation, for 10 s at 200 μA, in the right ventral amygdalofugal pathway; *B*, stimulation for 10 s at 100 μA on the left side of the medulla at a site just dorsal to the lateral edge of the pyramidal tract, approximately 5·5 mm caudal to stereotactic zero (atlas of Snider & Niemer, 1961).

caudally elicited what appeared to be one or two of the cardiovascular components of the defence response: these fragmentary responses, which were evoked at sites up to 1–2 mm deep to the ventral surface, were not traced further and the sites from which they were evoked are not illustrated in Fig. 4. Thus, the efferent pathway from the defence areas was traced as a narrow strip which runs through the medulla, ventral to the superior olive and the nucleus of the trapezoid body to the level of the rostral portion of the inferior olive where it lies ventral to the facial nucleus. We

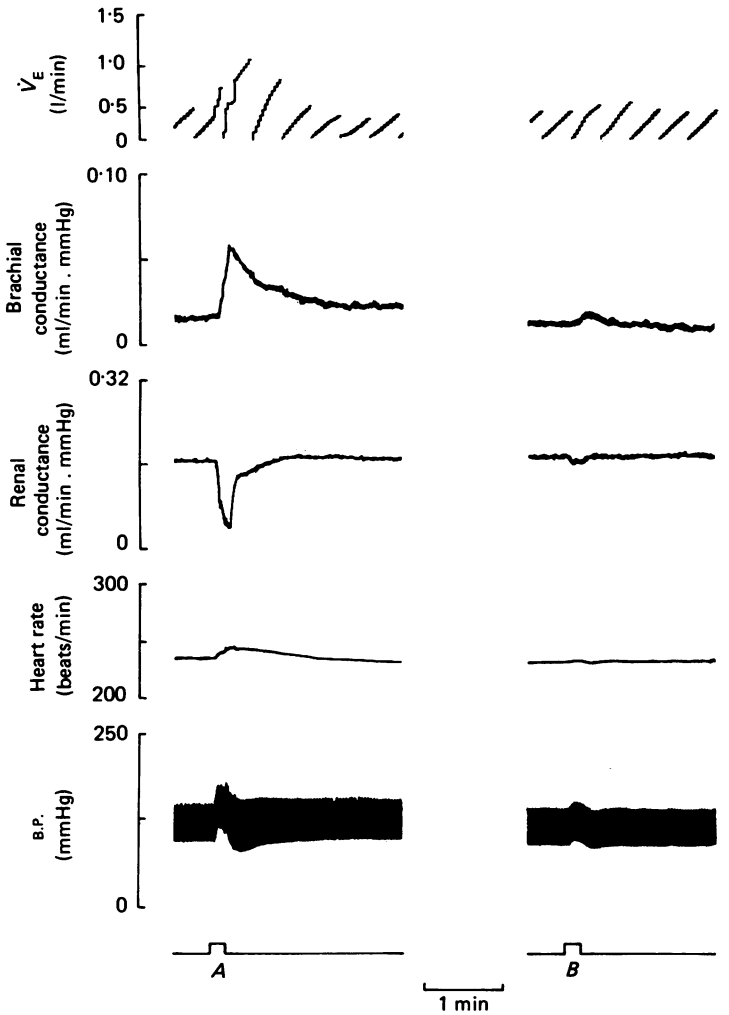

Fig. 3. Response evoked by stimulation within the efferent pathway from the defence areas and absence of response to stimulation slightly deeper in the medulla. Records, from below upwards, of arterial blood pressure (B.P.), heart rate, vascular conductance from left renal and left brachial arteries and respiratory minute volume ($\dot{V}_E$). Stimulus markers indicate two periods of stimulation, each for 10 s at 110 μA: *A*, at the ventral medullary surface on the left side and *B*, at a site 0·5 mm deep to the surface in the same penetration.

could not evoke the full pattern of response from sites caudal to this region and therefore could not trace the pathway further.

Stimulation at sites immediately lateral, medial or dorsal to this strip elicited little or no response (see Fig. 3). Stimulation at sites 1 mm or more lateral or dorsal to the strip, particularly in the caudal half of the area explored, usually evoked a marked pressor response with vasoconstriction in all vascular beds, including skeletal muscle.

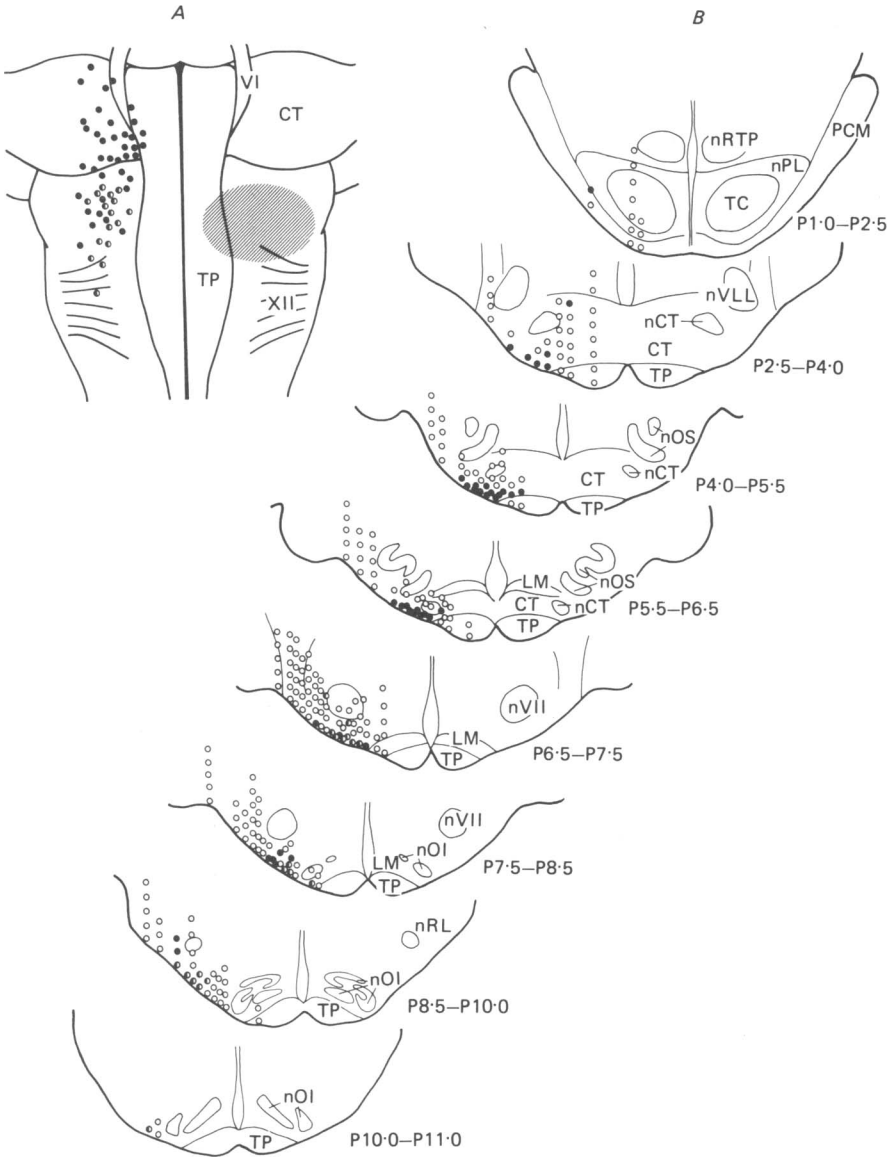

Fig. 4. Diagrammatic plan (*A*) and coronal sections (*B*) of the ventral medulla of the cat showing the sites at which stimulation evoked visceral alerting or similar responses, and the region to which glycine was applied. In *B*, sites at which stimulation evoked the full visceral alerting response are indicated by filled circles; sites which evoked responses similar to, but not identical to the visceral alerting response (see text) are indicated by half-filled circles; sites which evoked neither of these patterns of response are indicated by open circles. The sections show sites located at the levels indicated to the right of each section, expressed in mm caudal to stereotactic zero (atlas of Snider & Niemer, 1961). In *A*, filled and half-filled circles indicate the position of electrode penetrations in which stimulation elicited the above types of response. For clarity, all symbols are shown on the left side of the diagrams; the shaded area on the right side of *A* indicates the area over which glycine was applied. Abbreviations: CT, corpus trapezoideum; LM, lemniscus medialis; nCT, nucleus corporis trapezoidei; nOI, nucleus olivaris inferior; nOS, nucleus olivaris superior; nPL nucleus pontis lateralis; nRL, nucleus reticularis lateralis; nRTP, nucleus reticularis tegmenti pontis; nVLL, nucleus ventralis lemnisci lateralis; nVII, nucleus nervus facialis; PCM, pedunculus cerebellaris medius; TC, tractus corticobulbaris et corticospinalis; TP, tractus pyramidalis; VI, nervus abducens; XII, nervus hypoglossus.

The effect of lesions of the medullary strip

It was observed in several experiments that, after repeated electrical stimulation at a single site in the ventral medullary strip, the response to stimulation of the more rostral defence areas was somewhat reduced, the hind-limb vasodilatation ipsilateral to the site of the medullary stimulation being particularly attenuated. In each

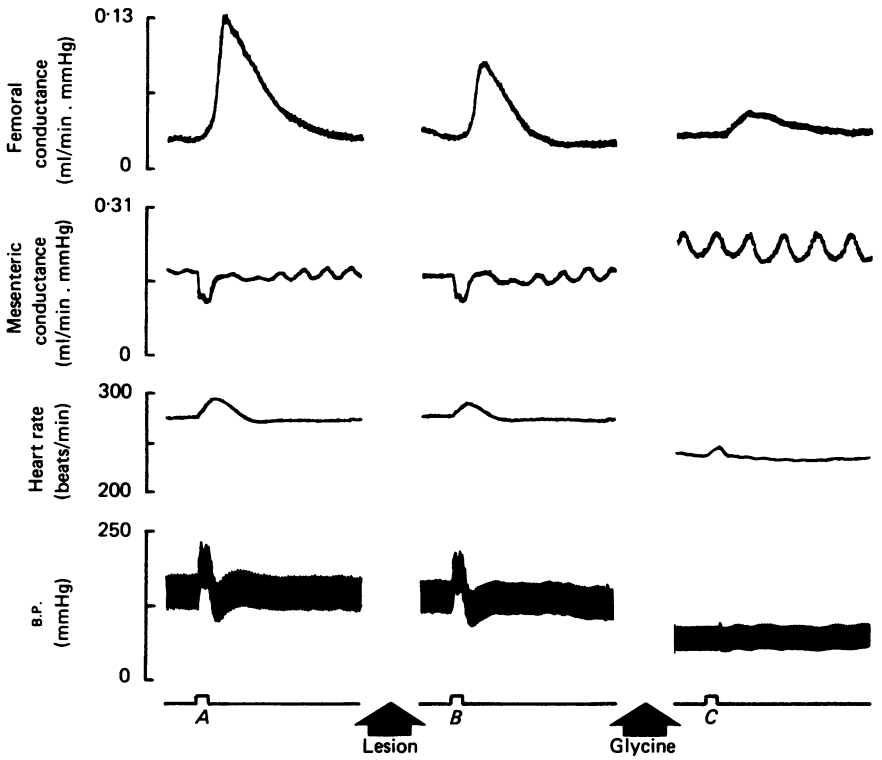

Fig. 5. Effect on resting cardiovascular variables and on the response to defence area stimulation of a ventral medullary lesion in the efferent pathway from the defence areas, and of subsequent application of glycine to the contralateral 'glycine-sensitive area'. Records, from below upwards, of arterial blood pressure (B.P.), heart rate, vascular conductance from the cranial mesenteric artery and the right femoral artery. Stimulus markers indicate three periods of stimulation, each for 10 s and 100 μA and each at the same site in the right ventral amygdalofugal pathway, *A*, before, and *B*, 2 min after a superfical radio-frequency lesion on the right side of the ventral medulla (see Fig. 6) and *C*, 5 min after the application of 10 μl glycine solution (200 mg/ml) to the 'glycine-sensitive area' on the left side of the ventral medulla.

experiment, this reduction was more pronounced after a radio-frequency lesion, made in the rostral part of the glycine-sensitive area on one side at a site previously shown to evoke the pattern of response characteristic of the alerting reaction. This is illustrated in Fig. 5. Each lesion was made from a ventral approach using an electrode whose tip just penetrated the surface. Microscopic examination of the serial sections of the medulla showed that this procedure resulted in an essentially cone-shaped

cavity in the ventral surface which was 0·5–1·0 mm in diameter and about 0·5 mm deep (Fig. 6). The area of functional disruption may have been somewhat larger, but this cannot be assessed from histological material. The effectiveness of the lesion in interrupting the efferent pathway was shown by the fact that no response could subsequently be evoked by stimulating normally effective sites in the pathway rostral

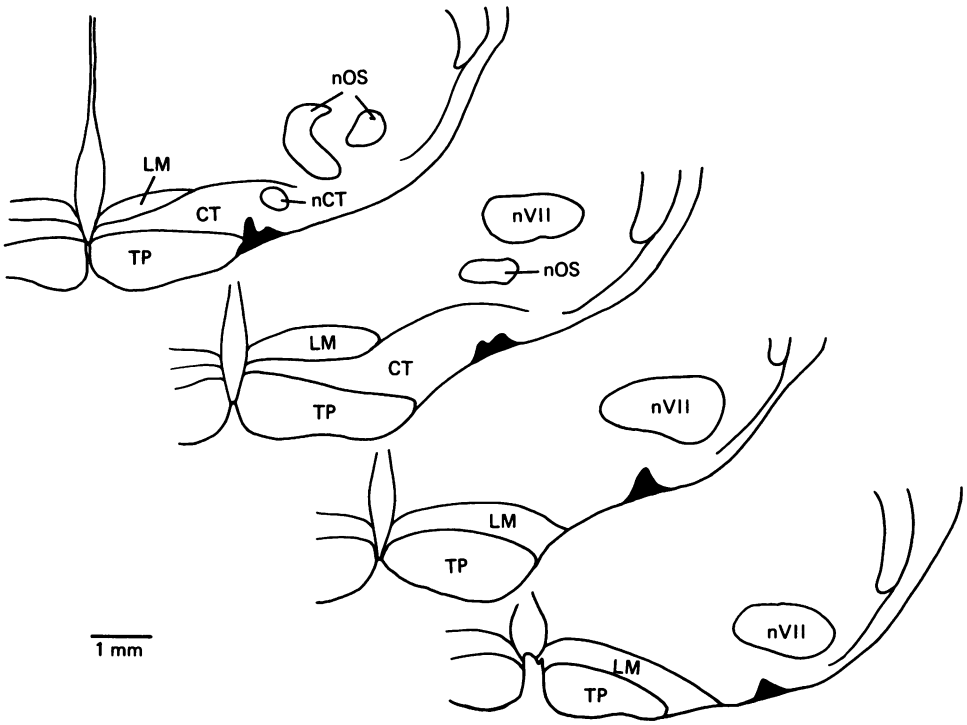

Fig. 6. Drawings from stained coronal sections through the medulla oblongata from each of four cats. Each drawing shows the visible tissue destruction (filled black) resulting from the passage of radio-frequency current via the stimulating electrode. Each lesion was roughly cone-shaped, and a section through the central (i.e. largest) part of the lesion is illustrated. The sections are arranged from rostral (upper section) to caudal (lowest section) and they represent levels from the caudal part of the superior olive to the central part of the facial nucleus (roughly P6·5–7·5, atlas of Snider & Niemer, 1961). The lesion which produced the effect shown in Fig. 5 is illustrated in the lowest drawing. Abbreviations as in Fig. 4.

to the lesion, while stimulation within the strip about 1 mm caudal to the centre of the lesion was still effective. Such a lesion, like the unilateral application of glycine, caused little change in the resting level of arterial pressure (Guertzenstein & Silver, 1974) heart rate, peripheral vascular conductance or respiration. Subsequent application of glycine, using a Perspex cup, but restricted to the side contralateral to the lesion, then produced the full effect which glycine would only have produced in the animal with an intact medulla if applied bilaterally: breathing ceased, arterial blood pressure dropped, and the test response from the defence areas was abolished or greatly reduced, as shown in Fig. 5. Subsequent application of glycine on the side

of the lesion, even flooding the exposed ventral surface of the brain stem with glycine, caused no further effect. Thus, in each of the four experiments, a small lesion restricted as far as possible to the efferent pathway from the defence areas was equivalent in its effect to that of glycine applied to that side of the medulla.

DISCUSSION

The efferent pathway for the visceral components of the alerting stage of the defence reaction ('visceral alerting response') has long been known to run from the hypothalamus and mid-brain defence areas through the ventral part of the brain stem, gradually approaching the ventral surface as it passes from the pons to the rostral medulla and thence to the caudal part of the medulla where it lies in a superficial position (Lindgren & Uvnäs, 1953; Lindgren *et al.* 1956; Abrahams *et al.* 1960; Schramm & Bignall, 1971). The mapping experiments which formed part of the present study have confirmed this location as a narrow strip running just lateral to the pyramidal tract, from the caudal extent of the pons to the level of the inferior olive. They provide a detailed map of its course within the medulla, showing that the pathway is relatively superficial from the level of the trapezoid body to that of the facial nucleus. Within, and just rostral to, the 'glycine-sensitive area' of Guertzenstein & Silver (1974) it comes particularly close to the surface, at least within 500 μm of it. This is consistent with the anatomical finding of Saper, Loewy, Swanson & Cowan (1976) that some fibres from the tuberal region of the hypothalamus do run in exactly this superficial location.

In our experiments, bilateral application of glycine to the sensitive area attenuated the effects of electrical stimulation of the defence areas of the amygdala, hypothalamus and mid-brain. The amino acid was effective in a concentration which acts only on neuronal cell bodies (Werman *et al.* 1968), so presumably it blocked the efferent pathway at a site of synaptic relay. As the response to unilateral defence area stimulation was only lost when the efferent pathway was blocked on both sides, some of the descending axons must cross the mid line rostral to the glycine-sensitive areas so as to activate the bulbospinal pathways bilaterally, as was concluded by Lindgren (1955).

There is a profuse penetration of the brain stem by blood vessels entering by the ventral surface of the medulla, some of them in the glycine-sensitive area (Cragg, Patterson & Purves, 1977; King, 1980), so drugs applied to the surface could possibly act on deeper structures. However, the effect of a superficial lesion of the ventral medulla which interrupted the efferent pathway was equivalent to that of glycine, which provides strong evidence that superficial structures are involved. In any case, to produce the block with glycine alone it is necessary that the drug be applied bilaterally and strictly to the special locus of sensitivity: flooding the medullary surface outside this restricted region has no such effect. The most reasonable conclusion is that the drug acts on neurones near the surface which are reached via the interstitial and perivascular spaces of the superfical layer of the ventral medulla, described in detail by Trouth, Odek-Ogunde & Holloway (1982).

In the mapping experiments, the pathway could not be followed much further caudal than the glycine-sensitive area. Although pronounced cardiovascular responses

could be evoked from sites caudal to this area, they did not comprise the full pattern of the visceral alerting response, some components being absent or even reversed, while in some tests changes in only one or two variables were evoked. This finding is consistent with the conclusion that the pathway is interrupted by a region of synapses at this level and suggests that the post-synaptic neurones in the glycine-sensitive area are dedicated to one or more, but not all components of the complex response pattern. This would be compatible with the finding of Loewy, Araujo & Kerr (1973) that increases in arterial blood pressure, bladder pressure or pupillary diameter could be separately evoked from sites in different medio-lateral positions in the caudal part of the medulla.

Of all the components of the visceral alerting response, the sympathetically mediated tachycardia, although reduced, was never abolished by glycine or lesions even in experiments in which the rest of the response pattern was effectively blocked. The evoked pupillary dilatation also persisted in some experiments. Thus, it is possible that some parts of the efferent pathway from the defence areas of the hypothalamus and mid-brain do not pass so superficially through the ventral medulla, or they may be interrupted by synapses which are not sensitive to glycine. There may even be other differences of pharmacological sensitivity related to the differences in functional dedication. For instance, McAllen *et al.* (1982) have recently reported that kainic acid applied rather diffusely to the ventral surface of the medulla can block pressor responses to hypothalamic stimulation without affecting the evoked pupillodilatation or retraction of the nictitating membranes.

Thus, apart from the exception just noted, the efferent pathway for the visceral alerting response is interrupted in a superficial region of the ventral medulla by glycine-sensitive synapses. There is already direct evidence of a relay in the pathway at this level. Donoghue, Hilton, Smith & Timms (1981) have reported that some superficial neurones in the more caudal part of the pathway we have defined, and within the glycine-sensitive area, receive a convergent input from the hypothalamic and mid-brain defence areas.

When glycine was applied bilaterally to the sensitive area it blocked transmission in the pathway *pari passu* with the reduction in arterial blood pressure. All our results, therefore, lead us to propose the following hypothesis: that glycine applied in this way reduces blood pressure by depressing the activity of the neurones which receive a tonic excitatory input from the defence areas of the hypothalamus and brain stem. These ventral medullary neurones may, of course, have other inputs or a capacity for spontaneous activity independently of any input; but, however their activity is maintained, we suggest that their effect on the relevant sympathetic outflows provides an essential background, in the absence of which other sympatho-excitatory inputs do not evoke sufficient vasomotor activity to maintain blood pressure at normal levels.

It is consistent with the results presented here that the input to the ventral medullary neurones which is most important in setting their level of activity arises from the defence areas, which are longitudinally arranged along the neuraxis, in the hypothalamus and brain stem (Hilton, 1975). This suggestion requires a reappraisal of the functional significance of the visceral alerting (defence) response. Hitherto, it has long been commonly held, as an extension of Cannon's original hypothesis (1929),

to be associated with acute episodes in daily life, when a sudden or novel stimulus evokes a dramatic behavioural episode, with a pattern of cardiovascular change appropriate to such an emergency reaction (Abrahams, Hilton & Zbrożyna 1960, 1964). However, there is already evidence that this cardiovascular response is graded with the strength of the stimulus, the same pattern occurring even during mild alerting (Caraffa-Braga, Granata & Pinotti, 1973). Seen in this light our findings lead us to suggest that the level of activity in the brain-stem defence areas necessarily associated with the waking state already engages the visceral alerting system to a certain extent, and that this level of engagement has an important part to play in setting the general level of arterial blood pressure. When glycine was applied in our experiments, the fall in total peripheral resistance seemed largely due to vasodilatation in the splanchnic area. Thus, our hypothesis implies that tonic descending activity from the visceral alerting system, including its relay in the ventral medulla, contributes significantly to the maintenance of arterial blood pressure, particularly through its constrictor effect on the splanchnic resistance vessels.

Since sleep must include a decrease in activity in the alerting system, it is consistent with our suggestions that, notably during paradoxical sleep, arterial blood pressure in the cat falls to a level similar to that we observed after bilateral application of glycine (Kumazawa, Baccelli, Guazzi, Mancia & Zanchetti, 1969) and that the pattern of cardiovascular change is a mirror-image of the alerting response (Mancia, Baccelli, Adams & Zanchetti, 1971). Moreover, Caraffa-Braga *et al.* (1973) have found, in the dog, that awakening is accompanied by the alerting pattern of cardiovascular response.

Apart from the effect on vasomotor tone, the visceral alerting system has a powerful excitatory effect on respiration, and blocking the relay in its descending pathway can result in apnoea. Much interest has centred in recent years on the concept of medullary chemoreceptors, largely as a result of the work of Loeschcke, Schlaefke and their co-workers. Areas of sensitivity to CO_2 and hydrogen ions have been localized to the ventral surface of the medulla, and neurones in the so-called 'area S' of Schlaefke & Loeschcke (1967), which corresponds closely to the glycine-sensitive area, have been said to act as an essential relay for central chemosensitivity and to provide the main central drive to respiration (Schlaefke & Loeschcke, 1967; Schlaefke, Kille & Loeschcke, 1979). However, the influence of area S is not restricted to the respiratory system, for bilateral cooling of area S has already been found to elicit a profound fall in arterial blood pressure (Hanna, Lioy & Polosa, 1979; Schlaefke & See, 1980; Millhorn, Eldridge & Waldrop, 1982), as has unilateral coagulation combined with contralateral cooling (Schlaefke & Loeschcke, 1967). Moreover, the results we are now reporting suggest that the modulation of ventilation by manoeuvres affecting this region of the medulla is, to some extent at least, non-specific, in that it is due to effects on transmission at synapses in the efferent pathway from the alerting areas. Thus, the normal activity in this pathway would provide a tonic excitatory input to respiration which may be exaggerated or reduced by intervention at synapses close to the surface of the brain stem. Interestingly, some workers who have supported the view that area S is specifically involved in central chemosensitivity have acknowledged that the apnoea and loss of sensitivity to carbon dioxide produced by bilateral cooling of 'area S' could equally well be explained by the interruption

of a tonic non-specific, excitatory drive to respiration (Cherniack, Euler, Homma & Kao, 1979; Millhorn *et al.* 1982). Our view is that 'area S' and the glycine-sensitive area are one and the same.

Lastly, the present results make it possible to re-assess the long-held view that there is a vasomotor centre in the caudal medulla (see Hilton (1981) for review). One of the ways of attempting to locate the centre has been to make lesions in the dorsal medulla, but even large lesions have usually led to small falls in blood pressure (e.g. Manning, 1965; Chai & Wang, 1968). In fact, Dittmar (1873) on whose early work the idea of a vasomotor centre is essentially based, had already shown that blood pressure was little affected by removal of the dorsal two-thirds of the medulla and he concluded that the area of special significance must lie in the ventrolateral reticular formation, near the facial nucleus. When Feldberg & Guertzenstein (1972) had shown that drugs applied to the ventral surface of the medulla can profoundly reduce the blood pressure, they raised once again the question of the significance of this region for vasomotor tone. Our proposal that the resting level of blood pressure may be determined largely by the ordinary level of activity within the alerting system, including its relays in the pathway we have defined in the ventral medulla, is compatible with all these results, without needing to postulate a vasomotor centre.

In conclusion, we propose that there is a group of relay neurones very near the ventral surface of the caudal medulla which forms an essential link in the efferent pathway from the alerting areas to the various autonomic effectors, and we already have some neurophysiological evidence to support this suggestion (Donoghue *et al.* 1981; Smith & Hilton, 1981). These neurones may also be essential for the maintenance of arterial blood pressure, though the question whether their state of activity depends entirely on the inputs to them, or whether they can be spontaneously active, must be left open at present.

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