Pulmonary Vasoconstriction Elicited by Stimulation of the Hypothalamic Integrative Area for the Defense Reaction

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ABSTRACT
Changes in the pulmonary circulation evoked by stimulation of the hypothalamic integrative area for the defense reaction were studied in 22 cats anesthetized with a chloralose-urethane mixture. Pulmonary arterial pressure and flow, left and right atrial pressures, aortic pressure, arterial flow to the skinned hind limb, and heart rate were all significantly increased. The calculated pulmonary vascular resistance was also significantly increased. The rise in pulmonary vascular resistance was abolished by bilateral stellectomy or by hexamethonium infusion; it was not affected by bilateral cervical vagotomy. Therefore, the increase in pulmonary vascular resistance was mediated by the thoracic sympathetic nerves. The increase in pulmonary arterial flow was mainly caused by an increase in heart rate; a small rise in calculated stroke volume also occurred.

ADDITIONAL KEY WORDS
pulmonary vascular resistance
left atrial pressure
sympathetic cholinergic vasodilation
heart rate stroke volume cat
thoracic sympathetic nerves
pulmonary arterial flow
pulmonary arterial pressure
stellectomy optic chiasm

In anesthetized cats, stimulation of the hypothalamus in the region of the fornix caused sympathetic cholinergic vasodilatation in skeletal muscle, vasoconstriction in skin and intestine, cardioacceleration and augmented cardiac contraction (1, 2). In conscious animals, threshold stimulation of this same area caused the cat to prick up its ears and dilate its pupils; skeletal muscle vasodilatation which was blocked by atropine accompanied the response (3, 4). Stronger stimulation provoked pilo-erection, unshleting of the claws, hissing and spitting, and running movements which could have caused flight or attack.

The term, defense reaction (Abwehrreaktion), was used by Hess (5) to refer to the integrated, behavioral aspects of this response. The results of Abrahams et al. (3, 4) showed that the hypothalamic region from which muscle vasodilatation could be elicited was identical with that which integrated the defense reaction.

The changes, if any, in the pulmonary circulation during the defense reaction are unknown. In addition, except for some careful experiments by Daly and Daly (6, 7), the functional significance of the pulmonary vasomotor nerve fibers is largely unknown (8). In the present experiments, we studied neurogenic changes in the pulmonary circulation elicited by threshold stimulation of the hypothalamic integrative area for the defense reaction. The calculated pulmonary vascular resistance was always significantly increased,
and this increase was mediated by the thoracic sympathetic nerves.

**Methods**

The experiments were done using 22 cats (2.5 to 5.0 kg) anesthetized with a chloralose-urethane mixture (60 mg/kg chloralose, Sigma Chemical Co.; 250 mg/kg urethane, Sigma Chemical Co.) injected iv after ether induction. The trachea was cannulated, positive pressure respiration was begun, and an incision was made in the fourth left interspace. The animals were paralyzed with gallamine triethiodide (Flaxedil, Davis and Geck), 2.0 mg/kg, injected iv; these doses do not interfere with sympathetic ganglionic transmission (9). The animals were allowed to recover from paralysis to insure that pinching the hind limb did not provoke a pain-like reaction. The stroke of the respiration pump was adjusted to maintain the end expiratory Pco₂ (measured continuously with an infrared CO₂ analyzer) at about 35 mm Hg which is normal for man in Salt Lake City (elevation 4500 ft). The arterial oxygen saturation measured by the Van Slyke method varied between 92 and 95%. Rectal temperature was maintained at 37°C by placing each cat on an electric heating pad. Polythene catheters were inserted into: (a) a femoral artery and passed retrograde to the main pulmonary artery. In 3 experiments, a catheter was passed distally along the lobular pulmonary artery until a pressure pulse similar to the left atrial pressure pulse was identified; however, blood samples could never be obtained from this site. To ensure that the pressures recorded from this site were not damped, the frequency response of the catheter-manometer system (flat ±5% to 20 to 25 cycle/sec) determined from a step input of pressure had to be unchanged. These pressures were called "wedged pulmonary arterial pressures." The catheters were connected to strain gauge manometers. The pressure pulse signal was passed through an RC network to obtain mean pressure.

Expired air was collected for 3 min in a small rubber bag, and the volume measured by passing the air into a 9-liter Collins Respirometer. This volume was corrected to STPD and the oxygen content was measured by the micro-Scholander technique. Oxygen content of aortic and pulmonary arterial blood was determined by the Van Slyke method. An electromagnetic flow probe (diameter 5 to 6 mm) was placed around the main pulmonary artery and connected to an electromagnetic flowmeter amplifier (Statham Module M-4001). The phasic flow signal was passed through an RC network to obtain mean flow. The probe was calibrated against the cardiac output obtained by the Fick method. Zero flow was assumed to occur at the end of diastole, i.e. at the bottom of the upstroke for pulmonary arterial flow. This zero corresponded to the amplifier baseline zero which showed no drift during the course of an experiment. Each flow transducer used in the present experiments was calibrated at least three times in vitro using segments of either pulmonary or external iliac artery, steady or pulsatile flow, and blood or saline. This was done to ensure that the output of each was linear over the range of flows encountered. The chest incision was covered with warm, saline-soaked gauze.

One hind limb was skinned from the groin to the ankle and the paw circulation occluded at the ankle by a tight ligature. The muscles were covered with warm, saline-soaked gauze and the skin was sutured over them. An electromagnetic flow transducer (diameter 1.0 to 1.5 mm), also connected to a Statham Module M-4001 amplifier, was placed around either the femoral or external iliac artery; zero flow was obtained by occluding the artery distal to the transducer and an in vitro calibration, using steady flow and blood or saline, was done at the end of each of the 18 experiments. The reliability of such calibrations in which the position of the transducer relative to the vessel was undoubtedly different from that in vivo, is unknown; however, such calibrations gave flow values similar to those obtained by Folkow et al. (10), who used a drop recorder. Records were made on a Honeywell Visicorder (Model 1508).

Stainless steel microelectrodes (tip diameter 2 to 5 μ) were made according to the method of Bishop and Collin (11), and insulated to the tip with Insl-X. The electrode resistance varied from 25 to 75 K ohm. The microelectrode was inserted by stereotaxic means into the perifornical region of the hypothalamus. The indifferent electrode was made the anode and placed in the frontal bone. The electrical stimuli were rectangular pulses, 2 msec in duration. Unless otherwise stated, the frequency of stimulation was 70/sec, and the duration of the train was 10 sec. Current was monitored continuously on an oscilloscope connected across a 1-Ω series resistor; slightly suprathreshold currents (0.8 to 1.5 ma) were used. When stimulation evoked both a maximal skeletal muscle vasodilatation, blocked by atropine (0.4 mg/kg injected iv), and a slight rise in arterial blood pressure, it was considered that the hypothalamic integrative area for the defense reaction had been located and an
optimal response obtained (1, 3). Subsequent histological confirmation (vide infra) was obtained in 8 experiments. No attempt was made to map the area as this had already been done (1, 4). The response could be elicited only at a distance within 1.0 mm or less in the sagittal, horizontal or coronal planes extending from that point which had yielded the optimal response.

At the end of 8 experiments, current (2.5 to 5.0 ma) was passed through the stimulating electrode for 60 sec. The brain was removed and fixed in 10% neutral formalin solution for 1 to 7 days. It was subsequently cut in a plane corresponding to the coronal plane of the stereotaxic coordinates. Histological localization was made on celloidin or paraffin-embedded sections stained with hematoxylin-eosin, Nissl’s and Weigert’s myelin stain.

In 3 experiments, bilateral cervical vagotomy was done; in 2 other experiments, bilateral stelllectomy was done. In 3 experiments, hexamethonium chloride (Hexameton, Burroughs Wellcome) in doses of 50 to 100 mg/kg per hr was injected iv using a Harvard constant infusion pump. These doses completely block nicotinic receptor sites of sympathetic ganglion cells (9). Atropine sulphate, 0.4 mg/kg, was injected iv in 6 experiments.

The following calculations were made:
- Pulmonary vascular resistance, \( \text{mm Hg/(ml/min)} = \frac{(\text{pulmonary arterial pressure} - \text{left atrial pressure})}{\text{pulmonary arterial flow}} \)
- Systemic vascular resistance = \( \frac{(\text{aortic pressure} - \text{right atrial pressure})}{\text{pulmonary arterial flow}} \)
- Femoral vascular resistance = \( \frac{(\text{aortic pressure} - \text{right atrial pressure})}{\text{femoral arterial flow}} \)

Heart rate was counted from the aortic pressure pulse over a 10-sec period, both before stimulation and during the peak response.

**Results**

Responses similar to that shown in Figure 1A were considered optimal and met our

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**Figure 1**

(A) Optimal response (see text) elicited by stimulation (signal on bottom reference trace) of the hypothalamic integrative areas for the defense reaction. Current = 1.0 ma. The response was analyzed at five stages labelled 1-5 (see text). Note that part 1 is the control. Abbreviations used in this and subsequent figures and Table 1 are: \( P_{PA} = \) pulmonary arterial pressures; \( P_{LA} = \) left atrial pressure; \( Q_{PA} = \) pulmonary arterial flow; \( PAO = \) aortic pressure; \( Q_{FA} = \) femoral arterial flow; and \( P_{RA} = \) right atrial pressure. The pressures and flows were recorded as mean values; all pressures are in millimeters of mercury and both flows are in milliliters per minute. (B) Effect of atropine sulfate (0.4 mg/kg) injected iv on the response. Note the striking reduction of femoral arterial flow and the larger, more sustained increase in aortic pressure. Pulmonary arterial pressure and flow showed changes similar to those in A.
### TABLE 1

**Effect of Hypothalamic Stimulation**

<table>
<thead>
<tr>
<th></th>
<th>2</th>
<th>4</th>
<th>5</th>
<th>Latency (sec)</th>
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<tr>
<td>PRA</td>
<td>2.8 ± 0.14</td>
<td>3.0 ± 0.16</td>
<td>3.5 ± 0.19</td>
<td>3.3 ± 0.16</td>
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<td>0.002</td>
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<td>&lt; 0.001</td>
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<tr>
<td>PLA</td>
<td>5.1 ± 0.22</td>
<td>5.6 ± 0.22</td>
<td>6.1 ± 0.28</td>
<td>5.8 ± 0.27</td>
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<tr>
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<tr>
<td>PPA</td>
<td>23.9 ± 0.58</td>
<td>26.4 ± 0.65</td>
<td>31.1 ± 0.71</td>
<td>29.9 ± 0.68</td>
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<tr>
<td>PAr</td>
<td>132.0 ± 2.3</td>
<td>162.6 ± 2.5</td>
<td>147.9 ± 3.2</td>
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<tr>
<td>QPA</td>
<td>357.1 ± 12.3</td>
<td>364.2 ± 13.0</td>
<td>456.2 ± 18.7</td>
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<td>QFA</td>
<td>11.8 ± 0.38</td>
<td>16.6 ± 0.76</td>
<td>37.3 ± 1.58</td>
<td>36.5 ± 1.73</td>
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<tr>
<td>PVR</td>
<td>0.005 ± 0.002</td>
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<td>0.058 ± 0.002</td>
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<tr>
<td>SVR</td>
<td>0.39 ± 0.02</td>
<td>0.48 ± 0.02</td>
<td>0.35 ± 0.02</td>
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<tr>
<td>FVR</td>
<td>11.6 ± 0.38</td>
<td>10.9 ± 0.44</td>
<td>4.3 ± 0.20</td>
<td>4.4 ± 0.21</td>
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<tr>
<td>HR</td>
<td>183.1 ± 5.9</td>
<td>210.0 ± 4.8</td>
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<tr>
<td>SV</td>
<td>1.9</td>
<td>2 ± 1</td>
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</table>

72 responses (mean ± SEM) from 11 experiments were analyzed at parts 1-5 (see Fig. 1). Parts 2-5 were compared with part 1 (control) using the pairing design method. *P* values are listed below the main entries. PVR = pulmonary vascular resistance; SVR = systemic vascular resistance; FVR = femoral vascular resistance; HR = heart rate (beat/min); and SV = stroke volume (ml/stroke). Other abbreviations as in legend to Figure 1. Latency was measured from the onset of stimulation to the onset of response. Units are those used in Figure 1.
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requirements for stimulation of the hypothalamic integrative area for the defense reaction (see Methods).

The optimal response shown in Figure 1A had five identifiable parts; these were compared statistically. 1 was the control. 2 was the peak of the initial, sharp aortic blood pressure rise which began after a latency of about 2 sec (see Table 1). The pulmonary arterial pressure always began to rise at this time; this rise always preceded the increase in pulmonary arterial flow. 3 was the peak of the femoral arterial flow increase which generally occurred at the same time as the peak of the pulmonary arterial pressure rise; the latency was 3.5 to 4.5 sec. 4 was the peak of the pulmonary arterial flow which occasionally occurred simultaneously with 3, but was always sustained over a longer time, and most often occurred after a longer latency of about 7.5 sec. 5 was the part occurring between 30 and 90 sec after cessation of stimulation; an additional 5 min often elapsed before the values returned to control levels.

Figure 2A is a plot of the calculated resistance values derived from the response shown in Figure 1A. Since the rise in pulmonary arterial pressure preceded the rise in pulmonary arterial flow, there was an initial increase in calculated pulmonary vascular resistance. This increase persisted during parts 3 and 4 despite the increase in pulmonary arterial flow, returning to control at part 5. The systemic vascular resistance, after a slight increase, fell during phases 3 and 4, and then returned to control. The femoral resistance fell, reached its nadir during 3 and 4, and then also returned to control.

Seventy-two similar responses were obtained in 11 experiments, and were analyzed statistically (Table 1). The remaining experiments were not included; either the response...
was not typical (5 experiments) or the experiments were concerned with the effects of vagotomy, stellectomy, hexamethonium infusion or exploration of the hypothalamus for other points from which the response could be evoked.

Table 1, column 1, shows the control values (mean ± SEM) in these 11 experiments. The values of pulmonary arterial flow and pressure were in agreement with those previously reported for the cat (12, 13); the values for the aortic pressure, and flow to the skinned hind limb were similar to those published by Eliasson et al. (1) and Folkow et al. (10). Right and left atrial pressures were similar to values in man. The absolute values elicited by hypothalamic stimulation are given in columns 2 to 5; these columns correspond to parts 2 to 5 of individual responses. Columns 2 to 5 were each compared statistically using the pairing design method with the control values in column 1; the \( P \) values are listed below the main entries. Hypothalamic stimulation elicited a statistically significant increase in every variable. During stage 5, aortic blood pressure, femoral arterial blood flow and femoral vascular resistance had returned to control; the remaining values returned to control over the following 4 to 5 min. Only the peak heart rate increase was calculated in these experiments; at these peak values, the calculated stroke volume was also increased.

In 3 experiments, the control "wedged pulmonary arterial pressure" was the same as the left atrial pressure. Hypothalamic stimulation provoked similar changes in both pressures.

![Graph](image-url)
In 5 experiments, the nictitating membrane and pupil were observed; in each instance retraction of the former and dilatation of the latter were provoked by hypothalamic stimulation.

In 18 of the 22 experiments, end-expiratory CO₂ was very slightly increased during hypothalamic stimulation; this increase followed the increase in pulmonary arterial flow.

The effect of atropine on the response of hypothalamic stimulation is shown in Figure 1B. The increases in pulmonary arterial pressure and flow were unaffected, but the great increase in femoral arterial flow was markedly reduced; the aortic blood pressure showed a larger, more sustained rise. The graph of the calculated resistances (Fig. 2) shows that the striking reduction in femoral vascular resistance before atropine was now reversed, but the increase in pulmonary vascular resistance was slightly greater. The systemic vascular resistance reflecting the larger, more sustained rise of aortic pressure was also increased. Similar effects of atropine were observed in an additional 5 experiments.

The effect of hexamethonium is shown in Figure 3. During infusion, hypothalamic stimulation no longer provoked any changes in

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**FIGURE 4**

A = coronal section at the level of the optic chiasm. Arrow indicates an electrolytic lesion in the hypothalamus at the site of stimulation. B = Nissl-stained section of hypothalamus. Arrow indicates the site of stimulation in the undifferentiated perifornical gray matter. OC = optic chiasm.
pulmonary arterial pressure or flow (Fig. 3B). The femoral arterial flow was now initially decreased, subsequently a small increase in flow accompanied by a slight drop in aortic pressure occurred. These changes were abolished after the intravenous injection of atropine (Fig. 3C). Neither pulmonary nor systemic resistances were altered by hypothalamic stimulation during hexamethonium infusion. The femoral vascular resistance, however, was now initially increased. The initial rise was followed by a drop and another rise in resistance. Such resistance changes were due to oscillations in femoral arterial flow (even while aortic pressure was constant) which disappeared gradually over 5 to 10 min. After atropine, these changes in femoral vascular resistance were abolished. Results similar to these were obtained in another 2 experiments.

Bilateral cervical vagotomy in 3 experiments had no effect on these responses. Bilateral stellectomy in 2 experiments prevented the changes in pulmonary arterial pressure and flow. However, the aortic pressure and the skeletal muscle blood flow increased as before.

In 7 out of 8 animals, the electrode tip was located in the perifornical, undifferentiated gray matter of the hypothalamus at the level of the optic chiasm or tuberal region. In 1 of these animals, there was a large electrolytic lesion that extended into the optic tract. In the remaining animal, the electrode tip was located in the tegmentum of the midbrain adjacent to the central gray matter. Similar results were reported by Abrahams et al. (4), (see their Figs. 4B, 4C, and 5). Our Figure 4A is representative of a coronal section of the brain at the level of the optic chiasm. The arrow indicates an electrolytic lesion in the hypothalamus at the site of stimulation. Figure 4B is a Nissl-stained section of the hypothalamus. The arrow indicates the perifornical site of stimulation.

Discussion

The present experiments show that stimulation of the hypothalamic integrative area for the defense reaction caused an increase in calculated pulmonary vascular resistance. The increase was abolished by bilateral stellectomy or by hexamethonium, but was unaffected by bilateral cervical vagotomy; therefore, the efferent arm was the thoracic sympathetic nerves. Since the "wedged pulmonary arterial pressure" and the left atrial pressure showed identical changes, the increased pulmonary vascular resistance must have been largely precapillary. However, the possibility that constriction may have occurred in the postcapillary capacitance vessels cannot be ruled out.

Although the increase in pulmonary vascular resistance was only 6 to 8% above control, it was associated with significant increases in pulmonary arterial flow and left atrial pressure both of which passively reduce the resistance in the distensible pulmonary vessels (8). The increased pulmonary vascular resistance was more sustained than the increased systemic resistance. The cause of the decrease in pulmonary vascular resistance during part 4 (Table 1) was not studied. The possibility that secretion from the adrenal medulla contributed to the increased pulmonary vascular resistance was considered unlikely since bilateral stellectomy abolished the increase; moreover, the latency (2 to 7.5 sec) of the major stages of the response was too short (2). In addition, Eliasson et al. (1) and Rosén (2) have shown that adrenal denervation or adrenalectomy did not alter the optimal response elicited by hypothalamic stimulation. In the early experiments when the stimulating current was greatly increased above threshold, a delayed rise in systemic and pulmonary blood pressures and in pulmonary flow appeared after 45 to 60 sec. Such changes may have been due to stimulation of the adrenal medulla.

It should be emphasized that in the present experiments, we assumed that the changes up to and including part 3 were due solely to hypothalamic stimulation, but that later stages were undoubtedly complicated by reflex adjustments initiated by the earlier response. Support for this view came from the experiments of Eliasson et al. (1) who showed that the initial stages of the response were...
not substantially altered by either sino-aortic denervation or prior occlusion of both common carotid arteries.

The possibility that the increase in pulmonary vascular resistance was due to precapillary anastomoses between the bronchial and pulmonary arteries cannot be ruled out because it was impossible during the surgical procedure to identify the bronchial arteries of the cat. This possibility was considered unlikely since the existence of such anastomoses in the cat has not been demonstrated (14). Transient, partial occlusion of the abdominal aorta that mechanically increased systemic, and therefore, bronchial arterial pressures, had no passive effect on pulmonary arterial pressure.

The influence of changes in bronchomotor tone was assumed to be negligible since Daly (15) has shown that the increased pulmonary vascular resistance evoked by stimulation of the thoracic sympathetic nerve trunk was not altered by passive effects due to concomitant bronchial constriction, bronchial dilatation, or changes in the bronchial circulation.

Although the behavioral aspects of the defense reaction were suppressed by the anesthesia used in the present experiments, the associated signs of cardiovascular sympathetic activity previously described (1-4), in particular, vasodilatation in skeletal muscle, were elicited. In addition to these signs, it is now clear that during excitation of the hypothalamic integrative area for the defense reaction, the pulmonary sympathetic nerves are stimulated to produce vasoconstriction.

The present experiments have also shown for the first time that stimulation of the hypothalamic integrative area for the defense reaction caused an increase in pulmonary arterial flow or cardiac output. This increase was largely due to the increase in heart rate but a small increase in stroke volume also occurred. It must be noted that the stroke volume was calculated at the peak heart rate response; earlier changes in heart rate and stroke volume were not determined.

The atropine-sensitive vasodilatation in skeletal muscle masked an element of vasoconstriction that was shown following injection of atropine (Figs. 1B and 2B). During hexamethonium infusion, the initial vasodilatation was also converted to vasoconstriction which was then abolished by atropine (Fig. 3). This would suggest that sympathetic ganglion cells giving rise to skeletal muscle cholinergic vasodilator fibers have predominantly nicotinic receptor sites (synaptic transmission blocked by hexamethonium alone), whereas the cells from which vasoconstrictor fibers originate have muscarinic receptor sites as well (synaptic transmission blocked by hexamethonium and atropine). In other experiments, direct recording from pre- and postganglionic sympathetic nerves supported this hypothesis (16).

References


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