Afferent Neural Pathway in the Regulation of Cardiopulmonary Responses to Tissue Hypermetabolism

CHANG-SENG LIANG, M.D., PH.D., AND WILLIAM B. HOOD, JR., M.D.

SUMMARY  We studied the role of neural transmission from hypermetabolic peripheral tissues in the regulation of cardiac output and pulmonary ventilation in chloralose-anesthetized dogs. Cross-circulation techniques with femoral-femoral or femoral-aortic anastomoses were used to produce a vascularly isolated, but normally innervated, hindlimb or lower half-body. 2,4-Dinitrophenol (DNP) was infused into the arterial side of the perfusion circuit to triple systemic arterial blood pressure increased, but neither heart rate nor pulmonary artery wedge pressure changed significantly. Pulmonary minute ventilation and arterial pH also increased, while arterial Pco2 fell. These changes were abolished when the nerve connections between the perfused limb and its parent body were severed. Normal saline, when administered in a similar manner, did not increase either ventilation or cardiac output, and simple denervation without previous infusions of DNP also had no effect. These results indicate that there are receptors sensitive to metabolic changes in the tissue, and that neural transmission is an important afferent link in regulating the cardiopulmonary responses to increased tissue metabolism.

BOTH CARDIAC output and pulmonary ventilation are increased during tissue hypermetabolism induced by 2,4-dinitrophenol (DNP). 1, 2 Levine and Huckabee 3 showed that the respiratory stimulation which follows DNP infusion is not a result of direct action of DNP on the brain or carotid chemoreceptors, hence the primary stimulus for these responses probably occurs in the peripheral hypermetabolic tissue from which it is transmitted to the central nervous system, heart, and lungs via neural or humoral pathways.

Earlier, Ramsay 4 infused DNP into the normally innervated hindlimb of a dog (recipient dog), which was separated vascularly from its own central circulation and perfused by a second dog. He found that ventilation of the recipient dog was stimulated after DNP infusion and that this response was abolished when the nerve connections between the limb and the body were severed. These findings suggest that a neural pathway is involved in the regulation of pulmonary ventilation during tissue hypermetabolism. These findings, however, were not confirmed by Bailen and Horvath, 5 who also used a hindlimb preparation but administered a smaller dose of DNP than did Ramsay. This difference in doses might explain in part the discrepancies between their experimental results. In addition, the mass of tissue that could be rendered hypermetabolic in the hindlimb preparation is small, and the magnitude of stimulus generated in such areas by the small dose of DNP used in the experiments of Bailen and Horvath might not have been sufficient to produce a significant increase in pulmonary ventilation.

In our present experiments, we attempted to study the role of neural transmission in the regulation of pulmonary ventilation and cardiac output during tissue hypermetabolism. In addition to a cross-perfused hindlimb preparation similar to that of Bailen and Horvath, we also used a preparation in which the lower half-body was cross-perfused and which allowed us to produce a larger mass of hypermetabolic tissue after infusion of DNP. Two doses of DNP were infused into the cross-perfused areas; the total amount exceeded that used by either of the previous two groups of investigators. 6, 7 Our results show that cardiac output and pulmonary ventilation can indeed be increased by a neural reflex caused by DNP-induced hypermetabolic changes in the peripheral tissue.

Methods

Adult dogs weighing between 16.5 and 28 kg were anesthetized with intravenous chloralose (60 mg/kg) after induction with vaporized methoxyflurane (Penthrane, Abbott Laboratories). The trachea was cannulated with a T-tube connected to a Benedict-Roth spirometer to record the rates of oxygen consumption and respiration and pulmonary minute ventilation. A femoral artery was cannulated with a Courmand catheter (No. 8 Fr.) and the main pulmonary artery with a Swan-Ganz catheter (No. 7 Fr.) (Edwards Laboratories, Inc., Santa Ana, Calif.) inserted through the right external jugular vein; both catheters were connected to Sanborn 267-AC pressure transducers and a Sanborn 7700 recorder to measure systemic arterial blood pressure, pulmonary artery wedge pressure, and heart rate. The “effective” pulmonary artery wedge pressure was calculated by subtracting from the measured pressure an...
intrathoracic pressure which was recorded through a PE 190 catheter inserted percutaneously into the thoracic cavity through the right 4th intercostal space. Another No. 8 Fr. catheter was inserted into a carotid artery and connected to a Gilford-Colson 103 densitometer to determine cardiac output* with indocyanine green (Cardio-Green). Mean systemic arterial blood pressure was measured by electronic integration and divided by cardiac output to obtain total peripheral vascular resistance. Heparin (30 U/kg) was administered intravenously to prevent clotting.

Cardiac output also was measured by the direct Fick principle.4 Blood pH, P02, and PCO2 were measured on a Radiometer PHM 71 acid-base analyzer. Blood samples were collected and analyzed for lactate7 and pyruvate.8 Blood oxygen content was analyzed with a Beckman GC-2A gas chromatograph.9 Blood oxygen capacity was determined by a cyanmethemoglobin method.10

In the cross-perfused hindlimb preparation, the hindlimb of a recipient dog, completely separated from its parent body except for the femoral and sciatic nerves, was perfused by the circulation of a second dog (donor) through femoral-femoral anastomoses. The femoral head was disarticulated and the free ends of excised muscle bundles were cauterized. Tygon tubing of a size selected to fit the caliber of the catheter inserted percutaneously into the thoracic cavity had been established, an interval of 30-45 minutes was allowed before the experiment was begun. Hindlimb blood flow was measured as the rate of venous outflow. This blood was infused into the donor dog by a peristaltic pump. Blood samples were taken for oxygen analyses and lactate and pyruvate measurements from arterial and venous sides of the perfusion circuit. The arteriovenous oxygen difference was multiplied by the regional blood flow to yield the perfusion circuit. The arteriovenous oxygen difference times blood flow.

Tissue hypermetabolism was induced in the cross-perfused hindlimb by two successive infusions of a neutral aqueous solution of 0.2% DNP separated by an interval of 20 minutes. The dose of DNP, for each infusion, was 2 ml/kg of donor weight and was administered into the arterial line supplying the isolated hindlimb over a 2-minute period. Twenty minutes after the second infusion, the femoral and sciatic nerves were severed. Hemodynamic measurements were made every 5 minutes and measurements of metabolism every 10 minutes in the recipient dog throughout the experiment. The results shown in Tables 1 and 2 were derived from the averages of two or three measurements made between 10 and 20 minutes after each infusion and denervation. In another group of animals prepared in the same way, normal saline (2 ml/kg) was infused and the same experimental protocol followed.

For the cross-perfused lower half-body preparation, the intestinal tract from duodenum to rectum was removed from a dog (recipient dog). The trunk wall was separated transversely into two parts at the level of L2-3. The only intact connections between these two halves of the body were the spine and ureters. The superior mesenteric artery was cannulated to measure systemic arterial blood pressure. The lower half-body was perfused, through a femoral-aortic anastomosis, by a donor dog. Blood from the inferior vena cava of the recipient was returned to femoral veins of the donor dog. Other details of the procedure were identical to those described for hindlimb perfusion. After cross-perfusion had been established, 4 g of 4-aminoantipyrine were infused into the arterial side of the perfusion circuit. Arterial blood samples were taken from the upper half-body of the recipient dog and from the donor dog 20 minutes later for determination of 4-aminoantipyrine.11 The amount of 4-aminoantipyrine present in the upper half-body of the recipient, in relation to that in the donor, was used as an index of vascular leakage between the upper and lower halves of the recipient dog. Circulatory and respiratory measurements were made in the recipient dog after successive infusions of normal saline (2 ml/kg) and two doses of DNP (4 mg/kg) into the perfusion circuit, spaced at 20-minute intervals.

The statistical significance of differences between experimental results in different groups of animals after normal saline or DNP infusions was determined by two-way analysis of variance for independent groups with repeated measures.12 If there was a significant difference among repeated measures, or in the group × measure interaction, Dunnett's test19 was used to determine the significance of differences between control and experimental values after DNP or normal saline infusion. The difference from the control was considered significant if P was less than 0.05. One-way analysis of variance was used to determine the significance of differences among repeated measures in the group of animals with cross-perfusion of the lower half-body. Correlation and regression coefficients were computed for the relationship between oxygen consumption and cardiac output, and the slopes of the regression lines in different groups were compared.14

Results

VASCULAR SEPARATION IN CROSS-PERFUSION PREPARATIONS

There were no vascular connections between the isolated perfused area and its parent body in the perfused hindlimb preparation, but such connections might exist via vertebral circulation in the cross-perfused lower half-body preparation. To determine the extent of vascular leaks through the vertebral circulation, 4 g of 4-aminoantipyrine were infused into the arterial side of the perfusion circuit prior to DNP infusion. Twenty minutes later, the plasma 4-aminoantipyrine concentration in the donor dog was 34.8 ± 3.3 mg/100 ml, whereas the concentration in the upper part of the recipient dog was 1.2 ± 0.2 mg/100 ml (n = 6), only 3.4 ± 0.7% of that in the donor dog. Because DNP is a vasodilator,15 greater vascular leaks might have been expected to occur after DNP infusion. Therefore, the same amount of 4-aminoantipyrine was administered into the perfusion circuit after DNP infusion. The plasma 4-aminoantipyrine concentration in the upper half-body of the recipient dog 20 minutes after administration was 1.4 ± 0.5 mg/100 ml (n = 5), again demonstrating the lack of significant vascular
leakage between the lower and upper half-bodies of the recipient dogs.

**EFFECTS OF DNP IN CROSS-PERFUSED AREAS**

Table 1 shows the effects of DNP on blood flow and tissue metabolism in the cross-perfused area. In both the cross-perfused hindlimb and lower half-body preparations, infusion of DNP produced increases not only in blood flow and oxygen consumption in the cross-perfused area, but also in anaerobic metabolism, as evidenced by increases in venous lactate concentration and lactate to pyruvate ratio. Lactate production also was increased in the cross-perfused area after DNP infusion. In contrast, none of these changes was seen after similar administration of normal saline (Table 1).

**HEMODYNAMIC AND VENTILATORY RESPONSES TO DNP INFUSION**

The circulatory and respiratory responses in the recipient dog to regional infusion of DNP are illustrated in Figure 1 and summarized in Table 2. For both hindlimb and lower half-body preparations, DNP infusion increased cardiac output and stroke volume. Pulmonary minute ventilation and respiratory rate also increased; as a result, arterial blood Pco₂ fell and pH rose. In the case of the cross-perfused lower half-body preparation, but not the cross-perfused hindlimb preparation, systemic arterial blood pressure increased significantly after DNP infusion; total peripheral vascular resistance, however, decreased slightly in both preparations. Heart rate and pulmonary artery wedge pressure did not change in either preparation. Arterial blood lactate and pyruvate concentrations, and the lactate to pyruvate ratio in the recipient dog, also did not change significantly after DNP infusion.

**Table 1 Effects of Infusion of 2,4-Dinitrophenol (DNP) on Blood Flow and Tissue Metabolism in the Cross-perfused Area**

<table>
<thead>
<tr>
<th></th>
<th>Blood flow (ml/min)</th>
<th>VO₂ (ml/min)</th>
<th>Venous [L] (mmol/liter)</th>
<th>Venous L/P</th>
<th>Lactate production (μmol/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Hindlimb cross-perfusion preparation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal saline infusions (n = 5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>51 ± 10</td>
<td>2.7 ± 0.4</td>
<td>3.89 ± 0.87</td>
<td>13.81 ± 1.47</td>
<td>30 ± 8</td>
</tr>
<tr>
<td>NS 1</td>
<td>44 ± 7</td>
<td>2.5 ± 0.3</td>
<td>4.91 ± 1.25</td>
<td>15.58 ± 2.27</td>
<td>36 ± 9</td>
</tr>
<tr>
<td>NS 2</td>
<td>39 ± 5</td>
<td>2.3 ± 0.2</td>
<td>5.35 ± 1.41</td>
<td>15.61 ± 2.30</td>
<td>33 ± 7</td>
</tr>
<tr>
<td>DNP Infusions (n = 7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>52 ± 6</td>
<td>3.6 ± 0.3</td>
<td>3.35 ± 0.43</td>
<td>13.87 ± 0.90</td>
<td>18 ± 5</td>
</tr>
<tr>
<td>DNP 1</td>
<td>85 ± 5*</td>
<td>10.5 ± 1.2*</td>
<td>7.50 ± 1.66*</td>
<td>33.82 ± 9.15</td>
<td>161 ± 37*</td>
</tr>
<tr>
<td>DNP 2</td>
<td>83 ± 9*</td>
<td>12.4 ± 1.6*</td>
<td>14.59 ± 0.90*</td>
<td>63.52 ± 13.25*</td>
<td>248 ± 56*</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Lower half-body cross-perfusion preparation (n = 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>154 ± 26</td>
<td>15.0 ± 2.7</td>
<td>6.51 ± 0.84</td>
<td>18.36 ± 1.63</td>
<td>85 ± 21</td>
</tr>
<tr>
<td>NS</td>
<td>152 ± 25</td>
<td>16.7 ± 3.3</td>
<td>6.99 ± 0.89</td>
<td>18.22 ± 1.33</td>
<td>83 ± 36</td>
</tr>
<tr>
<td>DNP 1</td>
<td>240 ± 42*</td>
<td>37.4 ± 7.0*</td>
<td>11.31 ± 1.42*</td>
<td>38.20 ± 7.16</td>
<td>659 ± 107*</td>
</tr>
<tr>
<td>DNP 2</td>
<td>287 ± 56*</td>
<td>41.6 ± 6.8*</td>
<td>18.36 ± 1.83*</td>
<td>77.08 ± 14.05*</td>
<td>486 ± 117*</td>
</tr>
<tr>
<td>ANOVA</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td></td>
</tr>
</tbody>
</table>

VO₂ = oxygen consumption; [L] = lactate concentration in blood water; L/P = lactate-pyruvate ratio; C = control; NS 1, NS 2 = after first and second infusions of normal saline (2 ml/kg); DNP 1, DNP 2 = after the first and second infusions of DNP (4 mg/kg); NS = after infusion of normal saline (2 mg/kg) (doses calculated on the basis of the weight of the donor dog).

Values are mean ± se. The number of experiments (n) is given in parentheses after each subheading. Analysis of variance (ANOVA) was used to determine the statistical significance of differences attributable to the sources of variation, i.e., measures (I), groups (II), and the group x measure interaction (III), at P < 0.05. Dunnetts test was used to determine the statistical significance of differences between control values and experimental values after each normal saline or DNP infusion.

* P < 0.05.
TABLE 2  Effects in Recipient Dogs of Infusion of 2,4-Dinitrophenol (DNP) into the Perfusion Circuit in Two Cross-perfusion Preparations

<table>
<thead>
<tr>
<th></th>
<th>V_{O_2} (ml/min)</th>
<th>Q (liters/min)</th>
<th>HR (beats/min)</th>
<th>SV (ml)</th>
<th>PA (mm Hg)</th>
<th>Pb (mm Hg)</th>
<th>pH</th>
<th>P_{CO_2} (mm Hg)</th>
<th>Ve (liters/min)</th>
<th>RR (per min)</th>
<th>L (mmol/liter)</th>
<th>P (mmol/liter)</th>
<th>L/P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>114 ± 10</td>
<td>2.95 ± 0.59</td>
<td>148 ± 15</td>
<td>20 ± 3</td>
<td>130 ± 10</td>
<td>44 ± 8</td>
<td>5.3 ± 0.5</td>
<td>7.37 ± 0.01</td>
<td>40.7 ± 2.9</td>
<td>5.58 ± 1.45</td>
<td>22.0 ± 8.6</td>
<td>2.97 ± 0.78</td>
<td>0.29 ± 0.06</td>
</tr>
<tr>
<td>NS 1</td>
<td>120 ± 13</td>
<td>2.46 ± 0.46</td>
<td>141 ± 13</td>
<td>18 ± 3</td>
<td>132 ± 10</td>
<td>54 ± 8</td>
<td>5.2 ± 0.6</td>
<td>7.37 ± 0.01</td>
<td>40.9 ± 3.6</td>
<td>5.66 ± 1.36</td>
<td>22.0 ± 8.6</td>
<td>2.88 ± 0.78</td>
<td>0.28 ± 0.06</td>
</tr>
<tr>
<td>NS 2</td>
<td>112 ± 22</td>
<td>2.43 ± 0.46</td>
<td>148 ± 11</td>
<td>16 ± 3</td>
<td>131 ± 9</td>
<td>54 ± 8</td>
<td>5.0 ± 0.7</td>
<td>7.35 ± 0.01</td>
<td>41.6 ± 3.5</td>
<td>6.05 ± 1.28</td>
<td>21.1 ± 7.7</td>
<td>3.25 ± 1.02</td>
<td>0.28 ± 0.06</td>
</tr>
<tr>
<td>D</td>
<td>140 ± 26</td>
<td>2.56 ± 0.51</td>
<td>148 ± 15</td>
<td>17 ± 2</td>
<td>137 ± 8</td>
<td>54 ± 11</td>
<td>5.0 ± 0.8</td>
<td>7.36 ± 0.01</td>
<td>39.1 ± 2.7</td>
<td>6.19 ± 1.57</td>
<td>20.2 ± 10.3</td>
<td>2.68 ± 0.66</td>
<td>0.25 ± 0.05</td>
</tr>
</tbody>
</table>

DNP infusions (n = 7; 24.7 ± 1.2 kg)

1. Hindlimb cross-perfusion preparation

Normal saline infusions (n = 5; 22.7 ± 1.4 kg)

Values are mean ± SE. The number of experiments (n) and body weights of the recipient dogs are given in parentheses after each subheading. Analysis of variance (ANOVA) was used to determine the statistical significance of differences attributable to the sources of variation, i.e., measures (I), groups (II), and the group x measure interaction (III), at P < 0.05. No roman numerals are given if there was no statistically significant difference. Dunnett's test* was used to determine the statistical significance of differences between control values and experimental values after each normal saline or DNP infusion.

* P < 0.05.

\[ V_{O_2} = \text{oxygen consumption}; \quad Q = \text{cardiac output}; \quad HR = \text{heart rate}; \quad SV = \text{stroke volume}; \quad P_a = \text{mean systemic arterial blood pressure}; \quad TPVR = \text{total peripheral vascular resistance}; \quad P_a = \text{mean pulmonary artery wedge pressure}; \quad pH = \text{arterial blood pH}; \quad P_{CO_2} = \text{arterial blood carbon dioxide tension}; \quad V_e = \text{pulmonary minute ventilation}; \quad RR = \text{respiratory rate}; \quad L = \text{litre}; \quad P = \text{millimol}; \quad L/P = \text{lactate-pyruvate ratio}; \quad C = \text{control}; \quad NS 1, NS 2 = \text{after the first and second infusions of normal saline (2 ml/kg)}; \quad D = \text{after derivation of the cross-perfused hindlimb}; \quad \text{DNP 1} = \text{DNP 2 after the first and second infusions of DNP (4 mg/kg)}; \quad \text{NS after infusion of normal saline (2 ml/kg) (doses based on the weight of the donor dog).} \]
Consequently, the increase in blood pH and decrease in cardiac output, stroke volume, and systemic arterial blood pressure toward preinfusion control values. We studied the role of neural transmission in mediating the responses of cardiac output and pulmonary ventilation to local DNP infusion by severing the femoral and sciatic nerves in the hindlimb preparation. Figure 1 and Table 2 show that surgical denervation after the second DNP infusion promptly lowered pulmonary minute ventilation to increment in oxygen consumption (AV \_\text{E}/P_{\text{CO}_2}) in these experiments was 128.6 ± 28.2; this, too, was much larger than that found for intact dogs (26.9 ± 1.8, f = 3.60, P < 0.001). The ratio of increment in pulmonary minute ventilation to increment in oxygen consumption (\Delta V_{\text{E}}/\Delta V_{\text{O}_2}) in these experiments was 128.6 ± 28.2; this, too, was much larger than that found for intact dogs (26.9 ± 1.8, t = 3.60, P < 0.001).

EFFECTS OF DENERVATION IN HINDLimb CROSS-PERFUSION PREPARATION

We studied the role of neural transmission in mediating the responses of cardiac output and pulmonary ventilation to local DNP infusion by severing the femoral and sciatic nerves in the hindlimb preparation. Figure 1 and Table 2 show that surgical denervation after the second DNP infusion lowered pulmonary minute ventilation and respiratory rate toward preinfusion control values. Consequently, the increase in blood pH and decrease in PCO\_2 which had been produced by DNP infusion were reversed. Denervation of the perfused hindlimb also reduced cardiac output, stroke volume, and systemic arterial blood pressure (Fig. 1, Table 2).

In contrast to DNP, normal saline, when similarly administered, produced no effect in the recipient dog (Table 2). Cutting the femoral and sciatic nerves without previous DNP infusions also did not produce any hemodynamic or respiratory changes in the recipient dog (Table 2).

Discussion

Separation of the circulation between the cross-perfused hindlimb and its parent body was complete in our cross-perfused hindlimb preparation. It was also adequate in the cross-perfused lower half-body preparation; 20 minutes after the inert, highly diffusible indicator 4-aminoantipyrine had been infused into the cross-perfusion circuit, the plasma concentrations of 4-aminoantipyrine in recipient dogs were less than 5% of those in donor dogs. It was estimated that the maximal amount of DNP that could have leaked into the recipient’s body was 0.4 mg/kg. This leakage of DNP is likely to be responsible for the circulatory and respiratory changes in our experiments, because this dose of DNP would be too small to produce any significant increase in cardiac output\textsuperscript{1} and because a dose of DNP of more than 2 mg/kg would have to be infused into intact dogs to produce increases in cardiac output and pulmonary ventilation of the magnitude reported for our present experiments.\textsuperscript{1,2} Furthermore, unlike the findings for intact dogs,\textsuperscript{1} in the cross-perfusion experiments the increase in cardiac output after DNP infusion was not accompanied by an increase in arteriovenous oxygen difference. In these cross-perfusion experiments the ratios of the increases in cardiac output and pulmonary ventilation to the increases in oxygen consumption were 4 to 5 times those found for intact dogs. Therefore, it seems likely that the increased cardiac output and pulmonary ventilation in the present experiments were not caused by tissue hypermetabolism in the recipient dog; this again confirms the absence of significant leakage of DNP into the recipient dog.

As expected, infusion of DNP increased oxygen consumption and blood flow in the cross-perfused area (Table 1). It also caused increases in tissue lactate production and lactate to pyruvate ratio, a finding which probably indicates relative tissue hypoxia.\textsuperscript{14} Table 2 shows that, in the cross-perfused lower half-body preparation, oxygen consumption increased slightly in the recipient dog after DNP infusion. This increase in the oxygen consumption in the recipient probably was caused by the cardiac and respiratory stimulation\textsuperscript{17} produced by local DNP infusion.

Control values for hemodynamic and metabolic parameters in the two groups of animals with cross-perfused hindlimbs were similar to those obtained for intact anesthetized dogs.\textsuperscript{1} However, in the cross-perfused lower half-body preparation, control values for cardiac output, stroke volume, oxygen consumption, and pulmonary ventilation were lower than those found for intact dogs; this probably was due to the fact that the recipient dogs, excluding cross-perfused areas, had smaller body masses. Both hindlimb and lower half-body preparations appeared stable during the experimental period because none of the variables measured changed significantly after infusion of normal saline (Table 2).

Our results show that cardiac output increased in the recipient dog after DNP was infused into the cross-perfused area which was connected to the body of the recipient dog only by nerves (Fig. 1, Table 2). This circulatory stimulation, which was correlated with the doses of DNP infused, was abolished when the cross-perfused area was denervated. Respiratory stimulation also occurred in the recipient dog after infusion of DNP into the perfusion circuit. Table 2 and Figure 1 show that pulmonary minute ventilation and respiratory rate increased in the recipient dog after infusion of DNP. Concomitantly, arterial PCO\_2 fell and pH rose. These respiratory changes, like the circulatory changes, were reversed after the nerve connections to the cross-perfused hindlimb had been cut.

The neural mechanism for respiratory stimulation after local DNP infusion was first reported by Ramsay,\textsuperscript{4} who used a cross-circulated hindlimb technique similar to ours, except that the vascular connections between the hindlimb
and body were interrupted by tourniquet rather than by surgery. His results, however, were not confirmed by Baillen and Horvath, who produced vascular isolation of the hindlimb from the body by surgical resection of muscle bundles and the femur. They suggested that the increase in pulmonary minute ventilation in Ramsay's experiments was caused by the stimulatory action of DNP that reached the body of the recipient dog through the blood supply of the femur. However, the latter investigators infused a dose of DNP of only 3 mg/kg into the donor dog, whereas Ramsay infused 5 mg/kg; this difference in doses might be responsible, at least in part, for their discrepant results. Our present study provides evidence that supports this hypothesis and reconciles both reports. In our hindlimb preparation, the limb was completely separated from the body, the only connection being the intact femoral and sciatic nerves. DNP, 4 mg/kg, was infused twice into the perfusion circuit. The amount of our first dose of DNP was between those used by the previous two groups of investigators, whereas the total amount which had been given after the second infusion exceeded that in the prior reports. Figure 1 and Table 2 show that infusion of DNP produced an increase in pulmonary minute ventilation, which, however, was not statistically significant until the second dose had been administered. Although the increase in pulmonary minute ventilation after the first infusion in our experiments and in the experiments of Baillen and Horvath was not statistically significant, the same directional changes were observed. In addition, we studied a lower half-body preparation in which larger tissue masses were rendered hypermetabolic by infusion of DNP. In this preparation, both doses of DNP caused a significant increase in pulmonary minute ventilation (Table 2). Our results clearly confirm Ramsay's earlier findings that DNP-induced metabolic changes can initiate a neurogenic reflex which is responsible for respiratory stimulation.

The neurogenic reflex originating from the hypermetabolic tissue undoubtedly plays an important role in the circulatory and respiratory responses to infusion of DNP. A similar neural reflex affecting respiration and circulation was demonstrated by Kao and Ray to arise from exercising limbs. It has been suggested that, during exercise, the stimulus for this reflex is chemical rather than mechanical in nature because the reflex responses are potentiated when circulation through the working muscle is blocked. Our experiments with DNP provide direct evidence that this neural reflex could, indeed, be brought about by the metabolic effects of exercise, independently of any increase in muscle movement. The "metabolic receptors" for this reflex are the free endings of small myelinated and unmyelinated fibers (groups III and IV).

Electrical stimulation of these afferent fibers from skeletal muscle produced hemodynamic changes similar to those that occur during exercise. These fibers enter the spinal cord and probably travel in the lateral spinal column, because the neurogenic hyperventilation caused by exercise in Kao's cross-circulation experiments may be abolished selectively by lateral chordotomy.

In summary, we have shown that an afferent neural mechanism plays an important role in the regulation of cardiopulmonary responses to tissue hypermetabolism. Our experiments, however, do not preclude a humoral mechanism, as in the work of Kao and Ray, nor do they contradict the possibility of cortical influences on the circulatory and respiratory responses in awake subjects, as in other studies.

Acknowledgments

We thank Adele Rymut and Barbara Kozol for expert technical assistance and Deborah Smith for her secretarial help. The indocyanine green (Cardio-Green) used in this study was kindly supplied by Hynson, Westcott & Dunning, Inc., Baltimore.

References

27. Goodwin GM, McCloskey DI, Mitchell JH: Cardiovascular and respiratory responses to changes in central command during isometric exercise at constant muscle tension. J Physiol (Lond) 226: 173-190, 1972
Afferent neural pathway in the regulation of cardiopulmonary responses to tissue hypermetabolism.
C S Liang and W B Hood, Jr

doi: 10.1161/01.RES.38.3.209

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/38/3/209