Platelet-activating factor modulates cardiorespiratory responses in the conscious rat

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Gozal, David, Gregory A. Holt, Gavin R. Graff, and José E. Torres. Platelet-activating factor modulates cardiorespiratory responses in the conscious rat. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R604–R611, 1998.—Platelet-activating factor receptor (PAFR) activation is associated with increases in neuronal excitability. We hypothesized that PAF may play a role in cardiorespiratory control. Ventilatory responses to microinjection of a long-acting PAF analog (mc-PAF, 1 µg in 1 µl) within the dorsocaudal brain stem were measured in unrestrained adult rats. mc-PAF elicited significant minute ventilation (Ve) enhancements that were primarily due to tidal volume increases and were accompanied by respiratory alkalosis, heart rate increase, and reduction of arterial blood pressure. Such cardiovascular and respiratory effects did not occur after administration of either vehicle or the inactive analog lyso-PAF. The effect was blocked when animals were coadministered the presynaptic PAFR antagonist BN-52021 or recombinant PAF acetyl hydrolase. To determine the relative contribution of PAF to hypercapnic and hypoxic ventilation, microinjections were performed in additional animals with either vehicle (CO, 1 µl) or with 5 µg in 1 µl of BN-52021. Hypercapnic challenges with 5% CO₂ were unaffected by BN-52021. In contrast, although 10% O₂ breathing increased Ve from 120.4 ± 7.5 to 204.6 ± 11.4 ml/min in CO, after BN-52021, Ve increased only from 118.7 ± 6.9 to 137.3 ± 8.9 ml/min (CO vs. BN-52021, P < 0.001). We conclude that PAFR activation in the dorsocaudal brain stem exerts significant cardioventilatory effects during normoxia and appears to play an important modulatory role in the Ve response to hypoxia in conscious rats.

respiratory control; brain stem; hypoxia; hypercapnia; blood pressure; heart rate

PLATELET-ACTIVATING FACTOR (1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine; PAF) is a potent phospholipid mediator that, in addition to platelet activation, displays a diverse array of biologic effects (3). The brain has the capacity to produce PAF by a de novo pathway involving cytidine diphosphate choline: 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine phosphotransferase that mediates the transfer of phosphocholine to alkylacyl-sn-glycerol (18). In the rat brain, this enzyme has a microsomal location and may be stimulated by neurotransmitters such as acetylcholine and dopamine to produce PAF in a Ca²⁺-dependent fashion (8, 12). There is now ample evidence that in vivo PAF synthesis occurs within particular brain structures during pharmacological manipulation of neural tissue. Indeed, PAF production will occur when acetylcholine (30), dopamine (8), or convulsant electrical stimuli (18) are applied. In addition, pathological conditions such as brain tissue ischemia or seizures are associated with significant PAF release, and the posts ischemic tissue damage is attenuated by PAF receptor (PAFR) antagonists (13, 26, 29), suggesting an important role for PAF as a mediator of neuronal injury.

In the central nervous system, PAFR appear to be predominantly localized in microglia, although neurons will also harbor PAFR (23). Such findings suggest the possibility that PAF-stimulated microglia may also be responsible, at least in part, for modulation of some of the physiological effects of PAF on neuronal activity. PAFR have also been shown to colocalize with N-methyl-D-aspartate (NMDA) glutamate receptors in hippocampal neurons (6), and PAF application exerts modulatory influences on neuronal differentiation, calcium fluxes, and long-term potentiation (LTP) (2, 10, 16, 17, 35). Furthermore, PAF may also act as a retrograde neural messenger (16), such that task-memory enhancements or their abolition was achieved in vivo by administration of a PAF analog and a synaptosomal PAF antagonist (BN-52021), respectively (14). When microsomal PAF antagonists such as BN-50730 were used, no inhibition of LTP occurred (16).

The nucleus of the solitary tract (NTS) in the dorsocaudal brain stem is the site of the first central synapse for primary afferent fibers originating from cardiopulmonary receptors, arterial baroreceptors, and chemoreceptors (15). PAFR are constitutively expressed in pontomedullary regions, where they display relative abundance (4–6). Thus, on the basis of aforementioned considerations, we postulated that PAF release in the dorsocaudal brain stem may mediate excitatory components of the ventilatory response to hypoxia.

In this study, we examined the cardioventilatory effects elicited by microinjection of a long-acting PAF analog (mc-PAF) to the dorsocaudal brain stem and assessed whether the presynaptic PAFR inhibitor BN-52021 and the accelerated degradation of active PAF by recombinant PAF acetyl hydrolase (rPAF-AH) modify cardiovascular and respiratory patterns. In addition, the effect of BN-52021 on the ventilatory responses to hypoxia and hypercapnia was also examined.

METHODS

Animals. The experimental protocols were approved by the Institutional Animal Care and Use Committee. Survival experiments were performed on adult male Sprague-Dawley rats (200–225 g). In a preliminary stage, anesthesia was induced by pentobarbital sodium (Nembutal, 50 mg/kg ip) and rectal temperature was monitored by a Harvard thermal probe (Harvard Apparatus, South Natick, NJ), with core temperature maintained at 37.5°C by a servocontrolled heating pad. A 1-cm incision of the skin at the groin was

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performed, and indwelling polyethylene catheters (PE-50; 0.56 mm ID; 0.88 mm OD) were surgically placed in the femoral artery and vein and advanced 4.5 cm to reach the abdominal aorta and inferior vena cava for subsequent blood pressure measurements, arterial blood sampling, and fluid or drug administration. After securing the catheters, these were tunneled subcutaneously, exteriorized in the dorsal aspect of the neck, flushed with a heparin-containing solution (1,000 U/ml saline), sealed with heat, and stored in a plastic cap stressed to the skin. Animals were then positioned in a stereotaxic apparatus (Kopf Instruments), and a small hole was drilled in the occipital skull. A cannula (22 gauge; Plastics One, Roanoke, VA) was then surgically implanted in the dorsocaudal brain stem according to the following stereotaxic coordinates: −13.85 mm from bregma, 0.2 mm off midline, and 7.3 mm depth (see Ref. 28). Adequate positioning of the cannula was verified on completion of the experiments by administration of a pentobarbital sodium overdose to the animal, followed by 1 µl microinjection of 20% methylene blue for histological assessment. After surgery, animals were allowed to recover for 48 h as demonstrated by return to normal feeding and activity schedules. Animals were provided with water and rat chow ad libitum and kept on a normal feeding and activity schedules. Animals were provided with water and rat chow ad libitum and kept on a.

Ventilatory and cardiovascular recordings. Cardiorespiratory measures were continuously acquired in the freely behaving, unrestrained animal placed in a previously calibrated 3-liter barometric chamber (Buxco Electronics, Troy, NY), using the methods described by Bartlett and Tenney (1) and Pappenheimer (27). To minimize the effect of signal drift due to temperature and pressure changes outside the chamber, we used a reference chamber of equal size in which temperature was measured using a T-type thermocouple. Environmental temperature was maintained slightly below the thermoneutral range (24°-28°C). A calibration volume of 0.5 ml of air was repeatedly introduced into the chamber, through which humidified air (90% relative humidity) was passed at a rate of 8 l/min using a precision barometric chamber (Buxco Electronics, Troy, NY), using the methods described by Bartlett and Tenney (1). The calibration volume was verified on completion of the experiments by administration of a pentobarbital sodium overdose to the animal, followed by 1 µl microinjection of 20% methylene blue for histological assessment. After surgery, animals were allowed to recover for 48 h as demonstrated by return to normal feeding and activity schedules. Animals were provided with water and rat chow ad libitum and kept on a normal feeding and activity schedules. Animals were provided with water and rat chow ad libitum and kept on a.

For normoxic baseline and challenge conditions. For experiments in which anesthesia was used, animals were anesthetized with isoflurane (2% by room air for 15 min). Anesthesia was maintained with 2% isoflurane in 95% room air and 5% CO2 for 15 min. After the anesthesia had been administered (5 µg in 1 µl of saline), the hypoxic challenge was repeated. Experiments were repeated 24 h later in the same animal. For BN-52021, a 2.5-fold higher concentration than that previously published for an in vivo study (5 µg in 1 µl of DMSO in saline, 20:80; see Ref. 14) was selected to ensure efficacy. After the optimal mc-PAF dosage was determined, cardiac and respiratory responses to mc-PAF were assessed in normoxia before and after pretreatment with BN-52021 (n = 9). To achieve this, 1 µg mc-PAF in 1 µl was microinjected and responses were assessed for 2 h. BN-52021 (5 µg) was then administered concurrently with the mc-PAF dose (1 µg) in a total volume of 1 µl.

In addition, a second group of seven animals was initially administered with vehicle (DMSO:saline), and 2 h later, with lyso-PAF (1 µg in 1 µl), the inactive analog, also dissolved in DMSO:saline (20:80). Finally, a third group of eight animals received mc-PAF, and 2 h later rPAF-AH at one of two possible doses (2.5 µmol in 1 µl (n = 4) or 5 µmol in 1 µl (n = 4)) was given concurrently with mc-PAF.

For PAFR antagonist experiments, an additional group of 13 rats was studied. Measurements were initially performed in room air to assess whether regional PAFR inhibition with BN-52021 in the dorsocaudal brain stem affected the normoxic cardiorespiratory patterns. Thirty minutes after BN-52021 or vehicle microinjection, hypoxic ventilatory responses were assessed by reducing the inspired oxygen fraction in the barometric chamber to 0.10 (baseline) and 0.05 (20 min) nitroge

Systemic arterial pressure was measured from the femoral artery catheter connected to a calibrated pressure transducer via a custom-designed swivel apparatus in the recording chamber (Buxco Electronics, Troy, NY). Physiological signals were digitized, and a beat-to-beat peak-trough analysis routine allowed computation of heart rate (HR) and mean arterial blood pressure (MAP).

Chemicals. Lyso-PAF, mc-PAF, and BN-52021 were purchased from commercial sources (Biomol, Plymouth Meeting, PA). rPAF-AH was kindly provided by Dr. Nicholas G. Bazan. Protocol. For determination of optimal dose responses, mc-PAF was dissolved in a mixture containing DMSO and normal saline (20:80) and slowly microinjected over a 1-min period at increasing concentrations ranging from 0.1 to 5 µg in 1 µl. For control, 1 µl of DMSO in saline (20:80) was microinjected 1 h before mc-PAF. Each microinjection was performed with the animal outside the barometric chamber, and on completion of the procedure the animal was returned to the recording chamber. One only dose was used for each animal, and cardioventilatory measures were monitored over a 2-h period postmicroinjection. It should be emphasized at this point that the potential contamination of our results by the presence of DMSO (20) was examined by additional experiments in five rats over two consecutive days. In these experiments, either 1 µl of saline or 1 µl of DMSO:saline (80:20) was microinjected in random order, and 30 min after microinjection animals underwent hypoxic challenges with 10% O2 for 30 min. Animals were then allowed to recover for 2–3 h, the other vehicle (1 µl of saline or 1 µl of DMSO:saline (80:20)) was then microinjected, and the hypoxic challenge was repeated. Experiments were repeated 24 h later in the sequence that had not been used the previous day. No significant ventilatory changes occurred after either solution during normoxia compared with preinjection values. Peak hyperventilation increases with hypoxia were 59 ± 6.8 and 54.9 ± 7.2% after saline alone and saline:DMSO, respectively (P not significant), suggesting that the presence of DMSO as used in all experimental conditions was unlikely to contribute to the experimental findings.
Ventilatory and arterial blood gas responses to mc-PAF and lyso-PAF in freely behaving rats

Microinjection of mc-PAF into the dorsocaudal brain stem caused dose-dependent \( V\dot{E} \) increases starting at 0.5 \( \mu \)g in 1 \( \mu \)l (\( P < 0.04 \), Fig. 1). We selected the dose of 1 \( \mu \)g in 1 \( \mu \)l for subsequent experiments because it was associated with highly reproducible and substantial \( V\dot{E} \) enhancements. Such ventilatory increases usually began 4–5 min after injection and lasted for a mean of 42.6 ± 3.6 min (range 31–49 min).

The ventilatory measurements associated with mc-PAF administration at the selected dose of 1 \( \mu \)g in 1 \( \mu \)l are shown in Table 1. The ventilatory enhancements elicited by mc-PAF were primarily dependent on VT increases (Fig. 2). In contrast, no ventilatory changes occurred when lyso-PAF was given (Table 1). The mc-PAF-induced ventilatory increases were absent when mc-PAF was concomitantly administered with either the presynaptic PAFR antagonist BN-52021 (Table 3). Indeed, \( V\dot{E} \) increased from 130.2 ± 9.4 ml/min in room air to 239.1 ± 12.9 ml/min during hypoxia in vehicle-treated animals and from 115.9 ± 11.1 to 198.3 ± 13.7 ml/min after BN-52021 treatment (\( P \) not significant). In contrast, BN-52021 markedly attenuated ventilatory responses to 10% \( O_2 \) in a separate group of seven animals (Table 4 and Fig. 2). Concordant with the ventilatory changes measured in the plethysmograph, significant increases in arterial blood pH and decreases in the partial pressure of \( CO_2 \) in arterial blood occurred in the mc-PAF-treated group only (Tables 1 and 2).

The next step in our experiments was to examine whether the presynaptic PAFR antagonist BN-52021 modified the ventilatory response to normoxia, hypoxia, and/or hypercapnia. When BN-52021 was administered, no significant \( V\dot{E} \) changes occurred during normoxia. However, although \( V\dot{E} \) was preserved, BN-52021 was associated with significant VT reductions and reciprocal increases in \( f \) (Fig. 3). Similar responses occurred with both doses of rPAF-AH (Table 2).

After BN-52021, the overall ventilatory response to hypercapnia was unaffected in six rats despite the changes in \( VT-f \) relationships associated with BN-52021 (Table 3). Indeed, \( V\dot{E} \) increased from 130.2 ± 9.4 ml/min in room air to 239.1 ± 12.9 ml/min during hypoxia in vehicle-treated animals and from 125.0 ± 7.1 to 144.9 ± 7.7 ml/min after BN-52021 treatment (\( P < 0.002 \)). The reduction in ventilatory response was associated with further VT decreases during hypoxia despite \( f \) increases (Table 4 and Fig. 3). Arterial blood gases paralleled ventilatory changes such that the magnitude of respiratory alkalosis during hypoxia was reduced after BN-52021 treatment (Table 4).

Cardiovascular responses. Administration of mc-PAF was associated with significant increases in HR (from

### Table 1. Ventilatory and arterial blood gas responses to mc-PAF and lyso-PAF in freely behaving rats

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Vehicle A</th>
<th>mc-PAF</th>
<th>Vehicle B</th>
<th>Lyso-PAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>( T_i ), s</td>
<td>0.222 ± 0.006</td>
<td>0.218 ± 0.007</td>
<td>0.192 ± 0.008</td>
<td>0.234 ± 0.005</td>
<td>0.212 ± 0.006</td>
</tr>
<tr>
<td>( T_e ), s</td>
<td>0.423 ± 0.015</td>
<td>0.415 ± 0.022</td>
<td>0.377 ± 0.017</td>
<td>0.501 ± 0.016</td>
<td>0.412 ± 0.021</td>
</tr>
<tr>
<td>VT, ml</td>
<td>1.41 ± 0.07</td>
<td>1.38 ± 0.08*</td>
<td>1.72 ± 0.11*</td>
<td>1.63 ± 0.05</td>
<td>1.46 ± 0.08</td>
</tr>
<tr>
<td>( f ), breaths/min</td>
<td>96.9 ± 3.5</td>
<td>99.1 ± 4.4</td>
<td>106.5 ± 4.3</td>
<td>81.9 ± 3.1</td>
<td>98.6 ± 5.9</td>
</tr>
<tr>
<td>( V_{TI} ), ml/min</td>
<td>132.7 ± 5.8</td>
<td>132.6 ± 6.3*</td>
<td>181.9 ± 15.9*</td>
<td>133.2 ± 5.6</td>
<td>143.8 ± 10.8</td>
</tr>
<tr>
<td>pH</td>
<td>7.430 ± 0.008</td>
<td>7.429 ± 0.007*</td>
<td>7.495 ± 0.014*</td>
<td>7.426 ± 0.012</td>
<td>7.428 ± 0.013</td>
</tr>
<tr>
<td>( P_{CO_2} ), mmHg</td>
<td>37.0 ± 0.5</td>
<td>36.9 ± 0.5*</td>
<td>30.6 ± 0.8*</td>
<td>36.8 ± 0.8</td>
<td>37.2 ± 0.8</td>
</tr>
<tr>
<td>( P_{O_2} ), mmHg</td>
<td>92.6 ± 0.6</td>
<td>92.6 ± 0.6</td>
<td>94.2 ± 0.5</td>
<td>91.6 ± 1.0</td>
<td>91.4 ± 1.2</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n = 14 \) for mc-platelet-activating factor (PAF) and \( n = 7 \) for lyso-PAF. \( T_i \), inspiratory time; \( T_e \), expiratory time; VT, tidal volume; \( f \), respiratory frequency; \( V\dot{E} \), minute ventilation; \( P_{CO_2} \) and \( P_{O_2} \), partial pressure of \( CO_2 \) and \( O_2 \), respectively, in arterial blood.

*\( P < 0.01 \), mc-PAF vs. vehicle A, ANOVA. Vehicle A, control conditions for animals receiving mc-PAF; vehicle B, control conditions for animals receiving lyso-PAF.
391.4 ± 11.8 beats/min after vehicle to 426.8 ± 10.3 beats/min, P < 0.001), which were prevented by treatment with BN-52021 and were absent when lyso-PAF was injected (Fig. 4). These changes in HR with mc-PAF coincided with the emergence of significant decreases in MAP (P < 0.001, Fig. 4), occurred within 6–8 min after injection, and lasted for 34.7 ± 6.6 min (range 18–46 min). Administration of lyso-PAF was not associated with marked ventilatory increases and primarily concerned withBN-52021 and were absent when lyso-PAF was injected (Fig. 4). These changes in HR with mc-PAF coincided with the emergence of significant decreases in MAP (P < 0.001, Fig. 4), occurred within 6–8 min after injection, and lasted for 34.7 ± 6.6 min (range 18–46 min). Administration of lyso-PAF was not associated with marked ventilatory increases and primarily significant decreases in MAP (P < 0.001, Fig. 4), occurred within 6–8 min after injection, and lasted for 34.7 ± 6.6 min (range 18–46 min). Administration of lyso-PAF was not associated with marked ventilatory increases and primarily MAP change, coinciding with the emergence of significant decreases in HR (394.3 ± 14.5 and 394.8 ± 11.5 beats/min, P not significant) or MAP (100.3 ± 5.7 and 102.0 ± 4.8 mmHg, P not significant).

When BN-52021 was microinjected in the dorsocaudal brain stem, MAP was not significantly affected although there was a trend for MAP increase (P = 0.057). During hypoxia, no significant differences in MAP before and after BN-52021 emerged (Fig. 5). In contrast, BN-52021 decreased HR (from 375.2 ± 9.0 to 331.3 ± 16.1 beats/min, P < 0.001) and the HR increase elicited by hypoxia was attenuated by BN-52021 (P < 0.04, Fig. 5).

DISCUSSION

The present study demonstrates that increasing PAF concentrations in the dorsocaudal brain stem are associated with marked ventilatory increases and primarily affect Vt rather than f. Furthermore, we show that local administration of a selective presynaptic PAFR antagonist into the dorsocaudal brain stem significantly attenuates the hypoxic ventilatory response without modifying the hypercapnic response. Thus our results suggest that changes in endogenous PAF release within the dorsocaudal brain stem may alter ventilatory pattern and may underlie important modulatory mechanisms of functionally relevant glial-neuronal interactions.

Several aspects of this work need to be addressed before proceeding with our interpretation of the experimental data. We selected the dorsocaudal brain stem as the target site for microinjection for several reasons. First, pontomedullary neurons express PAFR (4–6).

Table 2. Ventilatory responses to mc-PAF after pretreatment with BN-52021 or rPAF-AH

<table>
<thead>
<tr>
<th></th>
<th>Vehicle A (n = 9)</th>
<th>BN-52021 (n = 9)</th>
<th>mc-PAF + BN-52021 (n = 9)</th>
<th>Vehicle B (n = 8)</th>
<th>rPAF-AH (n = 8)</th>
<th>mc-PAF + rPAF-AH (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ti, s</td>
<td>0.188 ± 0.016</td>
<td>0.174 ± 0.011</td>
<td>0.183 ± 0.015</td>
<td>0.179 ± 0.016</td>
<td>0.164 ± 0.011</td>
<td>0.173 ± 0.016</td>
</tr>
<tr>
<td>Te, s</td>
<td>0.397 ± 0.018</td>
<td>0.376 ± 0.017</td>
<td>0.358 ± 0.022</td>
<td>0.359 ± 0.025</td>
<td>0.335 ± 0.024</td>
<td>0.361 ± 0.026</td>
</tr>
<tr>
<td>Vt, ml</td>
<td>1.32 ± 0.09</td>
<td>1.16 ± 0.11</td>
<td>1.11 ± 0.09</td>
<td>1.29 ± 0.11</td>
<td>1.08 ± 0.09</td>
<td>1.21 ± 0.13</td>
</tr>
<tr>
<td>f, breaths/min</td>
<td>102.5 ± 4.2</td>
<td>114.7 ± 4.0</td>
<td>116.9 ± 7.9</td>
<td>111.5 ± 4.7</td>
<td>120.6 ± 5.7</td>
<td>112.4 ± 7.7</td>
</tr>
<tr>
<td>Ve, ml/min</td>
<td>135.3 ± 7.8</td>
<td>125.7 ± 6.4</td>
<td>122.8 ± 7.0</td>
<td>143.8 ± 7.9</td>
<td>130.3 ± 9.0</td>
<td>136.0 ± 11.8</td>
</tr>
<tr>
<td>pH</td>
<td>7.423 ± 0.012</td>
<td>7.422 ± 0.013</td>
<td>7.419 ± 0.015</td>
<td>7.432 ± 0.015</td>
<td>7.430 ± 0.016</td>
<td>7.435 ± 0.017</td>
</tr>
<tr>
<td>PAO2, mmHg</td>
<td>37.4 ± 1.2</td>
<td>37.5 ± 0.8</td>
<td>37.3 ± 1.2</td>
<td>36.8 ± 1.3</td>
<td>37.2 ± 1.4</td>
<td>36.9 ± 1.6</td>
</tr>
<tr>
<td>Pao2, mmHg</td>
<td>94.6 ± 1.6</td>
<td>93.7 ± 1.1</td>
<td>93.9 ± 1.2</td>
<td>92.6 ± 1.8</td>
<td>90.3 ± 1.9</td>
<td>91.8 ± 1.7</td>
</tr>
</tbody>
</table>

Values are means ± SE. Note that data on recombinant PAF acetyl hydrolase (rPAF-AH) represent combined results of 2 doses because responses were virtually identical. *P < 0.02, BN-52021 vs. vehicle A; †P < 0.02, rPAF-AH vs. vehicle B; ‡P < 0.03, mc-PAF ± BN-52021 vs. vehicle A.
Second, one of the putative pathways for PAF activity as a retrograde messenger and neurotransmitter within the central nervous system involves the increased release of glutamate (10, 16, 35). Several lines of evidence indicate that glutamate release within the dorsocaudal brain stem in general, and more specifically within the NTS, which leads to activation of NMDA receptors, is critical for development of the hypoxic ventilatory response (7, 22, 25, 34). Third, the dorsocaudal brain stem is a relatively accessible site with demonstrated roles in cardiovascular and respiratory regulation, thereby offering a convenient target for the initial investigation of potential cardiopulmonary effects of PAF in freely behaving animals. However, it should be stressed that our experimental design does not allow for separation of effects that might be related to neuronal or glial sources. Similarly, it was not our intention to delineate the relative contributions of dorsocaudal brain stem nuclei to the various cardiovascular and breathing responses.

Ventilatory responses. Increased PAF activity within the dorsocaudal brain stem elicited increased ventilatory output in the conscious rat. Of interest, we consistently observed that such ventilatory changes were accompanied by reduced spontaneous motor activity and a state of quiet alertness, which were reminiscent of typical animal behavior during hypoxia. The ventilatory enhancements associated with mc-PAF administration were due to \( V_T \) increases with no changes in \( f \). Similarly, the attenuation of hypoxic ventilatory response by BN-52021 or rPAF-AH resulted from their marked effect on \( V_T \). The methods used in this study obviously preclude identification of neuronal populations in which activation or inactivation of PAFR leads...
to such Vt modification. However, we provide initial evidence that endogenous PAF release and PAFR activation are physiologically involved in tonically maintaining Vt as well as with Vt recruitment during hypoxia.

Such evidence is further strengthened by the similar effects of rPAF-AH and BN-52021 on ventilatory patterning. The biological activity of PAF depends on its structural features, namely, an ether linkage at the sn-1 position and an acetate group at the sn-2 position. The actions of PAF are abolished by hydrolysis of the acetyl residue, a reaction that is catalyzed by PAF acetyl hydrolase. Two forms of PAF acetyl hydrolase exist, one intracellular and the other that circulates in plasma. This enzyme has been cloned and displays structural features, namely, an ether linkage at the sn-1 position and an acetate group at the sn-2 position.

The actions of PAF are abolished by hydrolysis of the acetyl residue, a reaction that is catalyzed by PAF acetyl hydrolase. Two forms of PAF acetyl hydrolase exist, one intracellular and the other that circulates in plasma. This enzyme has been cloned and displays significant in vivo anti-inflammatory activity (33). We show here that rapid elimination of active PAF by rPAF-AH elicits Vt reductions and markedly attenuates mc-PAF-induced ventilatory effects.

The mechanism(s) underlying mc-PAF activity are yet to be fully elucidated. Kato and colleagues (16) showed that when a PAF analog was applied to CA1 postsynaptic neurons of the hippocampus, it elicited glutamate release from Shaffer collateral terminals. However, when glutamate and PAF were jointly applied to hippocampal neurons in culture, peak and steady-state glutamate currents were not modified, suggesting that PAF effects are probably mediated presynaptically (2). Furthermore, addition of PAF at concentrations similar to those found in stimulated brain tissue was found to induce LTP, provided that the participation of NMDA receptors was allowed (35). Conversely, when a PAF antagonist was introduced to an in vitro slice hippocampal preparation, LTP inhibition occurred in CA1 (10). We can only extrapolate from these findings on the mechanisms of PAF activity within the dorsocaudal brain stem. We speculate that basal PAF release is increased by tissue hypoxia within the dorsocaudal brain stem and leads to increased PAFR activation and glutamate release, thereby enhancing synaptic transmission. In support of such hypotheses is the attenuated ventilatory response to hypoxia that occurred when rats were pretreated with the PAFR antagonist BN-52021. In contrast, we found no evidence to support a functional role for PAF in central chemoreceptive responses.

Cardiovascular responses. Increases in PAF within the dorsocaudal brain stem elicited significant chronotropic effects as well as blood pressure reductions. Assuming that the major mechanism of PAF-mediated physiological effects involves the increase of glutamate release (16), our results are in agreement with previous studies examining the role of glutamate in cardiovascular control within this brain stem region. Indeed, L-glutamate microinjections in the dorsocaudal brain stem elicit decreases in blood pressure (7, 31, 32), and NMDA and non-NMDA glutamate receptors may mediate different components of these responses (19, 24). In conscious rats, antagonism of NMDA receptors in the NTS with AP-5 was without effect on resting arterial pressure and HR (9), suggesting that activation of cardiовagal pathways in the NTS may occur through NMDA receptors, whereas excitation of sympathoexcit-
atory pathways in the NTS may be mediated through non-NMDA receptors (9).

During hypoxia in the awake rat, arterial blood pressure either remained unchanged due to the balancing of an increased cardiac output with a vasodilatation in most systemic tissues (21) or actually decreased, as found in current experiments. These responses were not modified by PAFR blockade in the dorsocaudal brain stem, further suggesting that in the awake state blood pressure responses to carotid chemoreceptor stimulation do not appear to involve PAF-mediated mechanisms. In contrast, BN-52021 attenuated the chronotropic response to hypoxia (Fig. 5).

In summary, we provide initial evidence that changes in endogenous PAF release within the dorsocaudal brain stem exert an important modulatory role on cardiovascular and ventilatory functions. With respect to ventilatory patterning, PAF appears to be primarily involved in the regulation of Vt during both normoxia and hypoxia and does not modify the hypercapnic ventilatory drive. In addition, PAF will affect tonic blood pressure and HR characteristics during normoxia and will be associated with hypoxia-induced bradycardia rather than tachycardia without modifying the pressor responses to hypoxia. Further studies to elucidate the role of such novel neurotransmitters in cardiorespiratory control are currently underway.

Perspectives

The effects of PAF on ventilatory and cardiovascular regulation in regions of the brain stem further add to the currently emerging concept of the role of nonneuronal cells in determination of neuronal excitability and plasticity. In this context, powerful general stimuli (e.g., hypoxia) could lead to increased production and release of multiple biologically active substances such as PAF from both neuronal and nonneuronal cell populations. Such substances could in turn activate specific membrane-bound receptors in some of the very same and/or neighboring cells and thereby influence autocrine and/or paracrine loops of cell-cell interactions, modify gene activation patterns, and potentially lead to both immediate and long-term significant changes in neuronal excitability and synaptic transmission characteristics within a particular brain region.

Fig. 4. Mean ± SE heart rate (HR) and mean arterial blood pressure (MAP) at baseline (1) and changes occurring after dorsocaudal brain stem microinjections of vehicle (2, n = 9), mc-PAF (3, 1 μg in 1 μl, n = 9), presynaptic PAFR antagonist BN-52021 (4, 5 μg in 1 μl, n = 9), or mc-PAF and BN-52021 (5, n = 9). bpm, Beats/min. *P < 0.01, mc-PAF vs. other treatment; #P < 0.01, BN-52021 vs. other treatment.

Fig. 5. Mean ± SE HR and MAP changes in 7 rats after dorsocaudal brain stem microinjection of either BN-52021 (5 μg in 1 μl) or vehicle and subsequent hypoxic challenges (10% O2). *P < 0.001, BN-52021 vs. vehicle in normoxia; #P < 0.04, BN-52021 vs. vehicle in hypoxia.
REFERENCES