The action of platelet activating factor and its antagonism by WEB 2086 on human isolated airways

P.R.A. Johnson, C.L. Armour, J.L. Black

Dept of Pharmacology, University of Sydney, NSW, 2006, Australia.

Correspondence: P.R.A. Johnson, Dept of Pharmacology, University of Sydney, NSW, 2006, Australia.

Keywords: Human airways; in vitro; Lyso platelet activating factor; platelet activating factor; WEB 2086.

Received: July, 1989; accepted after revision August 29, 1989.

This study was supported by the Asthma Foundation of NSW and by the National Health and Medical Research Council of Australia.

Platelet activating factor (PAF, PAF-acether) is a naturally occurring phospholipid which has, in recent years, emerged as an important mediator in the inflammatory reactions which are associated with asthma [1]. PAF has been shown to induce bronchoconstriction in man, both in asthmatic and normal subjects, as well as causing prolonged bronchial hyperresponsiveness which can last for up to two weeks after exposure [2]. The mechanism of both these effects is unknown although it has been proposed that the PAF-induced bronchoconstriction may be dependent on the presence of inflammatory cells.

There are few studies of the direct effects of PAF on human airways in vitro [3–6], while no study has been made of the effects of PAF on human airways contraction induced by other agonists.

In order to obtain a better understanding of the in vivo PAF-induced changes within human airways, we have examined the direct contractile effects of PAF and Lyso PAF on human isolated airways as well as any PAF-induced alteration in smooth muscle contraction to electrical field stimulation, histamine, carbchol and KCl. In addition, WEB 2086, a potent and selective PAF antagonist [7, 8], was used to investigate the involvement of PAF receptors in both the direct contraction of human airway smooth muscle and any alteration in smooth muscle sensitivity induced by PAF.

Materials and methods

Human lung was obtained from specimens resected at thoracotomy as previously described [9]. Macroscopically normal tissue was supplied by the hospital pathologist and transported to the laboratory in Krebs-Henseleit solution (composition, mM: NaCl, 118.4; KCl, 4.7; CaCl2·2H2O, 2.5; MgSO4·7H2O, 1.2; KH2PO4, 1.2; NaHCO3, 25.0; and (+) glucose, 11.1) at 4°C that had been saturated with 5% CO2 in O2. Bronchi 2–3 mm in diameter were dissected free from surrounding parenchymal tissue and cut into either spiral strips 3×20 mm or rings 4–5 mm long. The tissue was then stored overnight in Krebs-Henseleit solution at 4°C. The following day, bronchial preparations were secured to tissue hooks while for the electrical field stimulation experiments bronchial rings were placed on custom made perspex tissue holders. Into the perspex tissue holders were inserted two stainless steel electrodes which were 3 mm apart and approximately 0.5 mm from the ring preparation. Tissues were suspended in 5 ml double jacketed organ baths under a load of 1 g. The load was continually adjusted to 1 g throughout the 1–3 h period of equilibration and then remained unaltered for the duration of the experiment. The bathing solution was maintained at 37°C, bubbled with 5% CO2 in O2 and exchanged every 20 min. Changes in tension were measured isometrically by means of Grass...
FTO3 transducers and recorded on a Grass 7P polygraph chart recorder. As there was no difference found in the responsiveness of the spiral or ring preparations, the results from the two types of preparation were combined.

To assess the direct effects of PAF, both cumulative concentration responses (CCRs) to PAF and responses to bolus doses of PAF were investigated. PAF was added in a cumulative manner to bronchial preparations at concentrations from 1×10^-4 M to 1×10^-3 M. Once the CCR was completed, the tissues were washed and allowed to return to baseline. A maximal contractile response to 10^-3 M carbachol was then obtained. In other experiments, bolus doses of 7×10^-8 or 7×10^-7 M PAF were added to bronchial preparations. Once the contraction had reached a plateau, the tissues were washed and allowed to return to baseline. A contractile response to carbachol (10^-3 M) was then obtained. In most cases only one dose of PAF was studied in each preparation, however in 8 preparations from 3 patients a second dose of PAF, at the same dose or higher (up to 1×10^-3 M), was studied. In a separate series of experiments, WEB 2086, 10^4 M, was added to half the tissues while the other half remained untreated. After 10 min, PAF, 7×10^-7 M, was added to both groups of tissues and once the contraction had reached a plateau or after a time period of 10 min, the tissues were washed until tone returned to baseline level. A contractile response to 10^-3 M carbachol was then obtained. The effects of bolus doses of Lyso PAF 7×10^-7, 1×10^-4 or 1×10^-3 M were studied in a similar manner to PAF.

The effect of PAF on induced muscle tone was investigated using histamine, carbachol and KCl. Initially, two successive CCRs to histamine were obtained in each tissue. Following completion of the first CCR, tissues were washed every 20 min until tension returned to baseline. Prior to the second CCR, half the tissues received PAF 7×10^-3 M while the other half received no pretreatment. The PAF was left in contact with the tissue for 5 min and then a second CCR to histamine was commenced in all tissues. After these initial experiments the effect of 7×10^-8 M PAF on repeated administration of bolus doses of histamine (10^-4-3×10^-4 M), carbachol (10^-4-10^-4 M) and KCl (10^-4-3×10^-4 M) was then investigated. In these experiments, bolus doses of agonist (a dose of agonist that gave between 20-60% of maximum contraction) were administered to four preparations from each patient. Once the contraction had reached a plateau the contraction was measured, the tissue was then washed and tone allowed to return to baseline levels. This process was repeated until three consecutive contractions varying not more than 10% from each other were obtained. PAF, 7×10^-4 M, was then added 30 s before the next addition of the same dose of the agonist under investigation. The contraction obtained to the agonist after PAF addition was measured and then compared to the mean of the three contractions prior to PAF addition. In a separate series of experiments, in half the tissues tested, WEB 2086, 10^-5 M, was added 10 min prior to PAF addition while the remainder had PAF alone. In control experiments, histamine bolus dose responses were studied in the presence of 0.1% bovine serum albumin (BSA) the diluent for the PAF) or WEB 2086, 10^4 M alone.

To assess the effect of PAF on electrical field stimulation (EFS)-induced contraction, bronchial rings were stimulated at constant parameters using Grass SD9 stimulators. The stimulation parameters (10-50 V, 6-12 Hz with a duration of 0.6 ms for 20 s) were such that a submaximal contractile response was obtained. To ensure that the EFS response was cholinergic and neurally mediated, the effects of atropine, 10^-7 M, and tetrodotoxin, 10^-4 M, were used initially to characterise the response. Following these initial experiments, in separate tissues, three consecutive EFS responses of similar magnitude (i.e. varying by not more than 10%) were obtained, and then PAF, 7×10^-3 M, was added and its effect on the subsequent EFS responses observed.

**Analysis of results**

The contractions to bolus doses of PAF (7×10^-8, 7×10^-7 M) were expressed both as a range of mg tension and as percentages of the carbachol maximal response. As PAF did not cause a contraction in every tissue, the proportion of tissues responding to PAF was also calculated. Weighted mean analysis [10] was used to calculate the overall mean and standard error of the mean (SEM) for the contraction to PAF, 7×10^-7 M, in the presence and absence of WEB 2086, 10^-3 M. This form of analysis was used to allow for the variation in contractile response to PAF within tissues from the same patient and between patients. The weighted mean values were then compared using a two-tailed Student’s t-test. A value of p<0.05 was considered significant.

In the experiments on the effect of PAF on induced muscle tone, the first and second CCR curves in the presence and absence of PAF, 7×10^-3 M, were compared. A mean value for the three contractions to each agonist or EFS prior to PAF addition was calculated and expressed as 100%. After PAF administration, the contraction to the agonist under investigation was expressed as a percentage of the mean value. In the potentiation experiments, PAF caused a direct contraction in 4 tissues, these results were omitted from the analysis. The percentage changes in contractile response after PAF addition were averaged from all tissues in each patient. A mean and SEM for each treatment was then calculated from all the patients. A two-tailed Student’s t-test was used to compare these values to the population mean of 100%. A value of p<0.05 was considered significant.

**Drugs and solutions**

Stock solutions of carbachol, histamine, KCl, WEB 2086, atropine and tetrodotoxin were made up in distilled water. The solutions were stored in 1 ml aliquots at -20°C and thawed as required. A stock solution of PAF was stored at -20°C in 2 mg·mL^-1 chloroform. Dilutions of PAF were freshly prepared on the day of the
experiment. An aliquot of PAF was removed from the stock solution, the chloroform allowed to evaporate and the remaining solid dissolved in 0.1% (w/v) BSA dissolved in normal saline. This solution was sonicated for 2 min to ensure that the PAF was completely dissolved, then kept on ice for the duration of the experiment. The BSA stock solution was stored at -20°C and thawed as required. A stock solution of Lyso PAF was made up in 10% ethanol in distilled water, and this solution was stored at -20°C in 0.2 ml aliquots. Dilutions of Lyso PAF were made in 0.1% (w/v) BSA. Dilutions of carbachol, histamine and KCl were made in Krebs-Henseleit solution and all dilutions were kept on ice for the duration of the experiment.

The following drugs were used: carbamylcholine chloride (carbachol; Sigma); histamine acid phosphate (Sigma); potassium chloride (KCl; Unilab); 1-O-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine (platelet activating factor (PAF); Sigma); 1-O-alkyl-sn-glyceryl-3-phosphorylcholine (Lyso platelet activating factor (Lyso PAF); Sigma); tetrodotoxin (Calbiochem); atropine sulphate (Drug Houses of Australia); 3-[4-(2-chlorophenyl)-9-methyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]-diazepin-2-yl]-1-(4-morpholinyl)-1-propanone (WEB 2086; Boehringer Ingelheim).

Results

Whereas there was no contraction observed when PAF was added in a cumulative manner, bolus doses induced a contractile response (fig. 1a, b). PAF, 7×10⁻⁶ M, caused a contraction in 8 of 56 tissues from 16 patients, while PAF, 7×10⁻⁷ M, induced a contraction in 37 of 42 tissues from 13 patients. The range of tension induced by PAF, 7×10⁻⁶ M, varied from 30-225 mg which was 2-55% of the carbachol maximum while the tension induced by PAF, 7×10⁻⁷ M, varied from 20-1,525 mg which was 3-72% of the carbachol maximum. The responses to PAF were found to be variable within tissue taken from the same patient. The number of tissues responding ranged from 1 of 3 to 6 of 6. In 8 tissues from 3 patients, a second dose of PAF added to the same tissue at the same dose or higher (up to 1×10⁻³ M and up to 2 h later) did not result in a second contraction. Lyso PAF, 7×10⁻⁷, 1×10⁻⁶ or 1×10⁻⁵ M, did not cause a contraction (fig. 1c).

In a separate series of experiments, WEB 2086, 10⁻⁶ M, reduced the frequency with which a contraction to PAF occurred, from 19 of 20 tissues from 5 patients (range 30-550 mg) to only 6 of 18 tissues from the same 5 patients (range 30-125 mg). The weighted mean for the contraction induced by PAF, 7×10⁻⁷ M, was 330±21 mg while in the presence of WEB 2086, 10⁻⁶ M, it was 18±8 mg. These values were significantly different (p<0.05, n=5).

PAF, 7×10⁻⁶ M, had no effect on the histamine CCR nor on the contractile responses to bolus doses of carbachol or KCl. However, it caused a significant potentiation of the contractile responses to bolus doses of histamine (fig. 2a). When compared to the control response of 100%, the response after PAF administration for carbachol was 101±5% (p>0.05, n=5 patients), KCl 105±17% (p>0.05, n=4) and histamine 125±7% (p<0.05, n=5). 0.1% BSA alone had no effect on the contractile responses to histamine. The PAF-induced potentiation of the histamine contractile responses was inhibited by WEB 2086, 10⁻⁶ M, (fig. 2b). When compared to the control response of 100%, the response after PAF was 138±16% (p<0.05, n=5), and in the presence of WEB 2086, 10⁻⁶ M, 97±7% (n=5). WEB 2086 alone had no effect on the contractile responses to histamine.

In preliminary experiments, it was found that the contractile responses induced by EFS were abolished by atropine, 10⁻⁷ M, and tetrodotoxin, 10⁻⁵ M. This indicates that the contractions were mediated via the release of acetylcholine from parasympathetic nerves. PAF, 7×10⁻⁶ M, did not significantly alter the EFS-induced contractile response. When compared to the control response of 100%, the response after PAF administration was 105±7% (p>0.05, n=4).
The mechanism of the PAF-induced contraction is unclear. The fact that Lyso PAF studied at similar or higher concentrations was unable to induce a contraction indicates that the contractile response to PAF is specific. In the present study we used WEB 2086 to investigate the specificity of the PAF-induced responses. This compound has been shown to inhibit other PAF mediated effects including bronchoconstriction in guinea-pigs, aggregation of human neutrophils [7] and enzyme release by human eosinophils [11]. The ability of WEB 2086 to block the response to PAF in our experiments indicates the involvement of PAF receptors. However, the location of the receptors is unknown. It is possible that the contraction induced by PAF was due to a direct action on PAF receptors on the smooth muscle. PAF receptors have been identified on the airway smooth muscle of guinea-pigs [12] as well as in human parenchymal tissue [13]. However, if PAF was acting at a receptor on the smooth muscle it would be expected that all tissues would respond to PAF. This was not the case, as some tissues from the same patient contracted while others failed to respond.

It was only possible to induce a contraction to PAF when it was administered in a bolus manner. With cumulative addition, it is possible that lower doses of PAF caused a desensitization of PAF receptors while being too low to induce a contraction. Desensitization has been reported previously in guinea-pig parenchymal strips [14] and lung parenchymal tissue from humans [6]. Desensitization may explain our inability to induce a second contraction to PAF in the same tissue.

It is possible that the contraction caused by PAF could be the result of the release of a secondary mediator capable of causing contraction of the airway smooth muscle. Thus PAF could be acting on receptors on the inflammatory cells within the lung tissue to cause the release of this secondary mediator. Specific PAF receptors have been identified on neutrophils [15] and platelets [16]. There is evidence for secondary mediator release in vivo. The bronchoconstriction to PAF observed in guinea-pigs is thought to be due to the release of lipoxygenase or cyclooxygenase products [17], while in man release of histamine is thought to be involved [18].

In the present study, the contractile response to PAF was variable both within and between patients. Variability in PAF responses has also been observed in vivo, suggesting an indirect mechanism of action [19, 20]. The response to PAF may depend on the release of mediators from inflammatory cells and it is possible that the number of inflammatory cells within a tissue may influence the response. It has been shown that the degree of inflammation within airway tissue varies both between and within lung samples [21]. It is possible that in some bronchial preparations, inflammatory cell numbers may have been too low to release enough secondary mediator to elicit a contraction. The fact that in the present study the PAF-induced contraction was not repeatable in the same tissue when the same dose or a higher dose was added, may provide further evidence for the release of a secondary mediator from inflammatory cells.

Our results contrast to those of previous investigators. In human isolated airway smooth muscle, CeRRINA et al. [3] and RAFFESTIN et al. [4] found that PAF did not induce a contraction, while SCHELLENBERG [5] found that the presence of platelets was mandatory for a PAF-induced contraction. There are a number of possible explanations for the differences between previous studies and the present investigation. Albumin is thought to be an important carrier for PAF [22]. It is also capable of causing an increase in the release of PAF from certain inflammatory cells [23]. Lack of use of albumin in some of the previous studies may have been the reason why a contraction to PAF was not observed. It is also possible that the patient group studied may have been different, or that the tissue sample size was too small. Thus the tissues used may have contained low levels of inflammation or even lacked inflammatory cells altogether, and when PAF was added insufficient quantities of a secondary mediator were released to induce a contraction.
The potentiation of histamine bolus dose responses by PAF in human airway smooth muscle observed in the present experiments appears to be due to an action on a PAF receptor as indicated by the blocking of the response by WEB 2086. Although the position of the receptor is unknown, it could be on the smooth muscle or on the inflammatory cells. It is possible that the potentiation of the histamine bolus responses may also be due to release of a secondary mediator. This secondary mediator may have a very short half life as PAF did not potentiate CCRs to histamine. Alternatively, since PAF was in contact with the tissue for the duration of the CCR it is possible that desensitization could occur to the potentiation response in a similar manner to the contractile response. In the guinea-pig, PAF has been shown to act on PAF receptors to enhance the responsiveness of isolated tracheal smooth muscle to K⁺ via the release of thromboxane A₂ [24]. Other mediators which are known to be released by PAF have the potential to alter airway smooth muscle sensitivity, such as leukotriene D₄ [25], prostaglandin F₂α and thromboxane A₂ [26]. It is also possible that PAF causes the release of endogenous acetylcholine in human airway smooth muscle as has been reported in dog trachea [27] and guinea-pig airway tissue [28]. However, if PAF was acting in this way in human airway muscle then it would be expected that PAF would enhance the contractile responses induced by EFS since this response is due to the release of acetylcholine. This did not occur in this study and therefore it is unlikely that PAF is acting via cholinergic mechanisms. Our results would confirm findings in vivo where PAF-induced bronchoconstriction was unchallenged by atropine [20].

The fact that PAF potentiates the contractile responses to bolus doses of histamine while having no effect on the responses to bolus doses of carbachol and KCl or EFS is interesting. In guinea-pigs, PAF was found to increase histamine responses in vivo while having no effect on the histamine contractility in vitro [29] whilst others have found that PAF caused an increase in the contractility of tracheal segments to K⁺ [24]. PAF also potentiates the KCl-induced contraction of porcine arteries while having no effect on noradrenaline contraction [30]. These studies indicate that any potentiating effect of PAF may be species, tissue and/or agonist dependent. In the study on pig vasculature, it was proposed that PAF may be having an effect on calcium influx [30]. We have previously shown that the calcium requirements of histamine, KCl and carbachol vary in human airway tissue [9]. The fact that PAF potentiated histamine responses and not those to carbachol or KCl may indicate that it alters the availability or source of calcium used in the histamine-induced contraction.

In summary, our results have shown that PAF causes a contraction of human isolated airways and that this contraction could be inhibited by WEB 2086. We also found that PAF, 7×10⁻⁸ M, increases histamine contractility while having no effect on the contractility of carbachol, KCl or endogenously released acetylcholine, and that this PAF-induced potentiation is also inhibited by WEB 2086. Further studies are needed to identify the mechanisms involved and to investigate whether the action of PAF may be different on asthmatic airways in vitro. These studies would lead to a better understanding of the role of PAF in airway hyperresponsiveness.

Acknowledgements: We are grateful to Boehringer Ingelheim for their kind donation of WEB 2086. We would like to thank the cardiothoracic surgeons from Royal Prince Alfred Hospital, The Repatriation Hospital, Royal North Shore Hospital and St. Vincent’s Hospital. The staff of the cardiothoracic theatres and pathology departments of the above hospitals provided invaluable assistance with the collection of the tissue.

References


RÉSUMÉ: Cette étude a investigué les effets directs et indirects du facteur activateur des plaquettes (PAF, PAF-acether) sur les voies aériennes isolées de l’homme. Le PAF a provoqué une contraction unique non répétitive, qui s’avère variable à la fois entre divers échantillons pulmonaires et entre divers tissus du même poumon. La marge de contractions induites par le PAF, administré à raison de 7x10^{-8} M, et de 7x10^{-7} M, est de 2-55 % et de 3-72 % de la réponse maximale au carbachol. L’antagoniste du PAF, le WEB 2086, dosé à 10^{-6} M, inhibe la contraction induite par le PAF à la dose de 7x10^{-7} M (p<0.05, n=5). Le PAF a provoqué une potentiation significative des effets constrictifs de doses d’histamine en bolus. Cette potentisation est inhibée par le WEB 2086 à la dose de 10^{-6} M (m=5, p<0.05). Ces résultats suggèrent d’abord que la bronchoconstriction observée chez l’homme après administration de PAF pourrait être le résultat d’une action du PAF sur le muscle lisse ou résulté de l’action d’un second médiateur libéré par le PAF à partir de cellules à l’intérieur du poumon. En deuxième lieu, l’hyperactivité bronchique observée après administration de PAF pourrait être due à une altération spécifique de la sensibilité des muscles lisses. Le fait que le WEB 2086 inhibe la réponse contractile au PAF et potentilise la contraction induite par l’histamine, pourrait suggérer que les récepteurs de PAF sont les médiateurs de ces réponses.

*Eur Respir J.*, 1990, 3, 55-60.