WEB 2086, A NOVEL SPECIFIC PAF ANTAGONIST, PREVENTS PAF INDUCED ARRHYTHMOGENICITY

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SUMMARY

The hetrazepine derivative WEB 2086 (0.5–2 mg/kg, i.v.), a well known PAF receptor antagonist, was found to reduce the arrhythmogenic effect of PAF in a dose dependent manner on ouabain induced arrhythmias in guinea-pigs. There was no detectable substance-specific influence of WEB 2086 on the threshold doses of ouabain, since in a dose of 2 mg/kg given i.v. it was able to inhibit cardiac rhythm disturbances induced by PAF as well. Guinea-pigs actively sensitized with ovalbumin responded to antigenic challenge with an increased susceptibility to ouabain induced arrhythmias. This arrhythmogenic effect could also be inhibited by WEB 2086.

In conclusion, WEB 2086 was found to exert marked protective effects against PAF related cardiac arrhythmias, indicating its potential usefulness for the treatment of cardiac anaphylaxis.

KEY WORDS: PAF, cardiac arrhythmias, ouabain, hetrazepines, anaphylaxis.

INTRODUCTION

Platelet-activating factor (PAF) characterized as an ether phospholipid is known to exert various pathophysiological effects in the sequelae of inflammation and anaphylaxis [1, 2]. Besides its relevance for anaphylactic lung reactions the important role of PAF for the mediation of cardiac anaphylactic symptoms has been postulated [3–5]. Cardiac effects of PAF include coronary flow reduction [4, 6], negative inotropic effect [6] and arrhythmogenicity [3, 7–9]. Additionally, blood pressure reduction [10, 11], extravasation and haemoconcentration [12, 13] belong to the pattern of PAF induced cardiovascular reactions. Recently, various PAF receptor antagonists of very different chemical nature have become available. A novel group among them are the hetrazepines possessing potent PAF antagonistic properties in vivo and in vitro [14, 15].

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The present study was designed to investigate the capability of WEB 2086 to inhibit cardiac arrhythmias induced by exogenous administration of PAF, ouabain and specific antigen in guinea-pigs.

**MATERIALS AND METHODS**

*Ouabain induced arrhythmia*

Guinea-pigs ranging in body weight from 300 up to 500 g, were anaesthetized with urethane (1·4 g/kg). There was no statistically significant difference between the various test groups concerning animal weight. The right jugular vein was cannulated using a polyethylene catheter. After recording a control ECG, PAF was applied intravenously in a dose of $10^{-11}$ mol/animal ($2·0-3·3 \times 10^{-11}$ mol/kg). This dose of PAF did not induce any change in blood pressure. A continuous infusion of ouabain (10 µg/kg/min) started 2 min later. WEB 2086 was given intravenously 2 min before the autacoid administration. The ECG was recorded continuously until the death of the animals using Einthoven II. The threshold doses of ouabain being necessary to induce ‘onset of arrhythmia’ (OA), ‘premature ventricular beats’ (PVB), ‘ventricular flutter’ (VF) and ‘ventricular fibrillation’ (FIB) were determined and expressed in µg/kg.

Thereby OA was defined as the onset of sinus arrhythmia and PVB as the first premature ventricular beat. VF was separated from ventricular tachycardia according to Zuckermann [16].

*PAF induced arrhythmia*

Guinea-pigs (300–500 g) were prepared as described above and PAF was administered in a dose of $2 \times 10^{-9}$ mol/animal ($4·5-6 \times 10^{-9}$ mol/kg) intravenously. This dose produced only slight changes in blood pressure, an increase followed by a decrease. These changes were not statistically significant. ECG alterations were followed until 10 min after giving the autacoid. WEB 2086 (2 mg/kg) was given 2 min before PAF. The incidence of OA and PVB were determined until 1 and 3 min after PAF injection, respectively. More severe arrhythmias could not be observed in this study.

*Influence of antigen induced mediator release on ouabain induced arrhythmia*

Guinea-pigs (300–500 g) were actively sensitized by the i.p. injection of 0·5 ml of saline containing 100 µg ovalbumin dispersed in 100 mg Al(OH)$_3$, modified according to [17].

After a 3-week period responders and non-responders were separated by an ovalbumin inhalation test using an ultrasonic nebulizer (1 mg ovalbumin/ml H$_2$O). One week later the anaphylactic reaction was triggered by an i.v. injection 0·1 mg/kg ovalbumin in responders. After 2 min ouabain infusion was started in order to determine the susceptibility of the guinea-pigs to arrhythmias after anaphylactic mediator release (see ouabain induced arrhythmia).

In preliminary experiments the dose of ovalbumin used as an antigen challenge was found not to be significantly in the influence on blood pressure or oxygen
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saturation as a consequence of anaphylactic bronchoconstriction. The $P_{O_2}$ value in venous blood was unchanged in non-sensitized and sensitized animals by giving $0.1 \text{ mg/kg}$ ovalbumin i.v. ($d_i = 0.4 \pm 0.1 \text{ kPa}$, $N_S$ and $d_i = 1.0 \pm 0.8 \text{ kPa}$, $N_S$, respectively). In this way haemodynamic or broncho-obstructive alterations could be excluded. However, a higher dose of ovalbumin ($2 \text{ mg/kg}$, i.v.) decreased significantly the $P_{O_2}$ value ($d_i = -2 \pm 0.2 \text{ kPa}$, $P<0.001$) in sensitized guinea-pigs as a result of bronchoconstriction.

**Drugs used**

The drugs used were ouabain, WEB 2086 (Boehringer Ingelheim, FRG) and PAF (Novabiochem, Clery-en-Vexin, France).

PAF was used in a buffer solution ($8.5 \text{ g NaCl}, 0.2 \text{ g KCl}, 0.2 \text{ g KH}_2\text{PO}_4$, $1.44 \text{ g Na}_2\text{HPO}_4\cdot\text{H}_2\text{O}$ per litre distilled water, $\text{pH} = 7.4$) containing $2.5 \text{ mg/ml human serum albumin.}$

The buffer solution of PAF served as control.

Ovalbumin (Sigma, St Louis, USA) and the adjuvant Al(OH)$_3$ (Merck, Darmstadt, FRG) were applied for making up the antigen suspension.

**Statistics**

Results were expressed as the mean $\pm$ SEM for each group. Statistical comparison was made using the Conservative Approximation of Newman–Keuls test for ouabain induced arrhythmias and $\chi^2$-test for PAF induced arrhythmias. Results with $P<0.05$ are considered to be statistically significant.

**RESULTS**

PAF in a dose of $10^{-11}$ mol/animal ($2.0\times10^{-11}$ mol/kg) decreased the threshold doses of ouabain induced arrhythmia to $43.2 \pm 3.0$ (control $57.0 \pm 3.0$) for OA ($P<0.05$), $80.6 \pm 2.6$ (control $89.6 \pm 2.7$) for PVB ($N_S$), $100.4 \pm 4.0$ (control $119.9 \pm 3.1$) for VF ($P<0.05$), and $105.6 \pm 2.6$ (control $126.4 \pm 3.5$) $\mu g/kg$ for FIB ($P<0.05$) (Fig. 1).

The PAF antagonist hetrazepine WEB 2086, used in a dose of $2 \text{ mg/kg}$, intravenously, was able to abolish completely the arrhythmogenic effect of PAF. It increased the threshold dose of ouabain compared to PAF up to $61.0 \pm 3.8$ for OA ($P<0.05$), $85.1 \pm 6.4$ for PVB ($N_S$), $135.8 \pm 8.1$ for VF ($P<0.05$) and $144.9 \pm 14.5 \mu g/kg$ for FIB ($P<0.05$) (Fig. 1).

After $1 \text{ mg/kg}$ WEB 2086 PAF induced arrhythmogenicity was reduced less markedly. It changed the threshold dose of ouabain to $50.7 \pm 4.4$ for OA ($N_S$), $90.0 \pm 8.3$ for PVB ($N_S$), $135.1 \pm 4.1$ for VF ($P<0.05$) and $142.5 \pm 5.3 \mu g/kg$ for FIB ($P<0.05$).

However, $0.5 \text{ mg/kg}$ WEB 2086 was devoid of any protective effect under these experimental conditions (not shown).

The effects of WEB 2086 on PAF induced arrhythmogenicity showed a clear dose-dependency as demonstrated for fibrillation (Fig. 2). WEB 2086 given in doses of $0.5$, $2.0 \text{ mg/kg}$ or $10 \text{ mg/kg}$ showed no substance-specific antiarrhythmic effect on ouabain induced arrhythmia (Table I).
Fig. 1. The influence of WEB 2086 on PAF induced arrhythmogenicity in ouabain induced arrhythmia in guinea-pigs. *P<0.05.

Fig. 2. The influence of WEB 2086 on the PAF effect, demonstrated for ouabain induced fibrillation. *P<0.05.

After intravenous administration of PAF (2×10^-9 mol/animal or 4.56×10^-9 mol/kg, 7/10 animals showed sinus arrhythmia during the first minute. This could be reduced to 2/10 after pretreatment with 2 mg/kg WEB 2086. The incidence of premature ventricular beats was 6/10 in the PAF group and could be reduced to 1/10 in WEB 2086 treated animals. These protective effects of WEB 2086 were statistically significant for sinus arrhythmia with P<0.05 (Fig. 3).
Influence of WEB 2086 on the threshold doses of ouabain induced arrhythmia in guinea-pigs (in µg/kg ouabain ± SEM, n values in parentheses)

<table>
<thead>
<tr>
<th>Drug</th>
<th>OA</th>
<th>PVB</th>
<th>VF</th>
<th>FIB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>62.5±4.8 (9)</td>
<td>102.5±6.0 (10)</td>
<td>126.5±6.8 (9)</td>
<td>140.4±7.0 (6)</td>
</tr>
<tr>
<td>WEB 2086</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 mg/kg i.v.</td>
<td>53.7±4.1 (10)</td>
<td>88.6±5.3 (9)</td>
<td>123.2±6.2 (7)</td>
<td>143.5±8.4 (7)</td>
</tr>
<tr>
<td>2.0 mg/kg i.v.</td>
<td>57.0±2.4 (8)</td>
<td>88.2±3.8 (7)</td>
<td>122.8±5.5 (8)</td>
<td>131.4±4.5 (7)</td>
</tr>
<tr>
<td>10.0 mg/kg i.v.</td>
<td>58.2±3.1 (8)</td>
<td>90.5±6.1 (7)</td>
<td>123.1±5.2 (7)</td>
<td>136.4±4.4 (8)</td>
</tr>
</tbody>
</table>

Fig. 3. The influence of WEB 2086 on PAF induced arrhythmia in guinea-pigs. *P<0.05.

In actively sensitized guinea-pigs an antigen challenge caused a marked decrease in threshold doses of ouabain to 41.9±2.1 (control 54.8±2.5) for OA (P<0.001), 59.1±2.6 (control 79.4±2.8) for PVB (P<0.001), 89.3±4.2 (control 123.1±7.0) for VF (P<0.001) and 100.8±4.7 (control 138.2±7.5) µg/kg for FIB (P<0.001) (Fig. 4).

The antigen doses used did not affect other relevant parameters as tested before. The arrhythmogenic effects of anaphylactic mediators released during this procedure could be counteracted by WEB 2086 in a dose-dependent manner.

While 2 and 5 mg/kg WEB 2086 given intravenously could only reduce the susceptibility to arrhythmias for OA/PVB and OA/FIB, respectively, 10 mg/kg was able to abolish completely the arrhythmogenic action (Table II, Fig. 4).
Table II
Influence of WEB 2086 on the anaphylactic mediator induced arrhythmogenicity in ouabain induced arrhythmia (in μg/kg ouabain ± SEM, n values in parentheses)

<table>
<thead>
<tr>
<th>Drug</th>
<th>OA</th>
<th>PVB</th>
<th>VF</th>
<th>FIB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline/ovalbumin</td>
<td>41·9 ± 2·1 (15)</td>
<td>59·1 ± 2·6 (18)</td>
<td>89·3 ± 4·2 (19)</td>
<td>100·8 ± 4·7 (18)</td>
</tr>
<tr>
<td>WEB 2086 2 mg/kg i.v.</td>
<td>54·6 ± 3·0 (10)***</td>
<td>69·0 ± 3·9 (14)*</td>
<td>98·2 ± 6·0 (15)</td>
<td>114·3 ± 6·7 (15)</td>
</tr>
<tr>
<td>WEB 2086 5 mg/kg i.v.</td>
<td>50·8 ± 2·7 (13)*</td>
<td>66·6 ± 2·8 (13)</td>
<td>98·5 ± 4·9 (14)</td>
<td>115·8 ± 4·3 (14)*</td>
</tr>
<tr>
<td>WEB 2086 10 mg/kg i.v.</td>
<td>57·8 ± 3·0 (11)***</td>
<td>72·3 ± 4·1 (13)***</td>
<td>110·0 ± 6·6 (13)**</td>
<td>125·7 ± 6·4 (13)***</td>
</tr>
</tbody>
</table>

*P<0·05; **P<0·01; ***P<0·001.

**Fig. 4.** The influence of WEB 2086 on the threshold doses of ouabain after ovalbumin induced mediator release in actively sensitized guinea-pigs. **P<0·01; ***P<0·001.

**DISCUSSION**

As reported earlier [7, 18], PAF was able to decrease significantly the threshold doses of ouabain induced arrhythmias signalizing arrhythmogenic properties. This is in accordance with other reports characterizing PAF as an arrhythmogenic factor [3, 4, 8, 9]. The observed arrhythmogenic effect could be due to a direct electrophysiological influence [19, 20], a PAF mediated release of leukotrienes and thromboxane [18], or PAF induced oxygen-centred radical formation [21].
On the other hand, there is increasing evidence that PAF could also be involved in arrhythmogenesis after ischaemia and reperfusion [5, 22]. PAF antagonists can prevent such kind of arrhythmias in a different manner [8, 9, 23]. The PAF antagonist WEB 2086 was capable of counteracting PAF induced arrhythmogenicity in ouabain induced arrhythmias in a dose-dependent manner, confirming the results with BN 52 021 obtained by using the same method [18].

If PAF is applied intravenously without ouabain OA and PVB could be observed, mostly during the first minute and within 3 min after PAF application, respectively. The dose of PAF used in this study produced only a slight increase followed by a small decrease in blood pressure which were not at the level of statistical significance. These direct arrhythmogenic PAF effects could be prevented by WEB 2086. For the incidence of sinus arrhythmia only, this protective effect of WEB 2086 was confirmed by statistical analysis. In addition to these methods using PAF applied exogenously, a release of endogenous PAF was provoked by an ovalbumin challenge in actively sensitized guinea-pigs. This anaphylactic mediator release was studied on the threshold doses of ouabain.

WEB 2086 in doses of 2 and 5 mg/kg i.v. could only diminish arrhythmias, while 10 mg/kg abolished the arrhythmogenic action of the mediators released. In conclusion these results suggest that the arrhythmogenic potency of mediators released during anaphylactic reactions is mainly due to PAF. However, an important role for histamine and serotonin in cardiac anaphylactic symptoms cannot be excluded, especially because WEB 2086 is also known to reduce the anaphylactic histamine release in actively sensitized guinea-pigs [24]. Our findings suggest that the antiarrhythmic effect of WEB 2086 observed under our experimental conditions must be due to its PAF antagonistic activity, because WEB 2086 alone failed to influence ouabain induced arrhythmias.

Direct CNS related effects can be excluded as a mode of action [25]. Hetrazepines, e.g. WEB 2086, are known to be effective PAF antagonists as demonstrated to inhibit cardiovascular responses induced by PAF in guinea-pigs [24]. Systemic hypotension produced by PAF could be inhibited by hetrazepines also in rats [15]. Furthermore, PAF induced lethality in mice, where toxic cardiovascular effects are thought to play a major role, is antagonized by these PAF antagonists [26].

In conclusion, our findings demonstrate that the PAF antagonist hetrazepine WEB 2086 prevents PAF induced cardiac rhythm disturbances. This novel PAF antagonist is effective against exogenous by administered PAF as well as endogenous PAF formation during anaphylactic reactions.

Thus, hetrazepines, like WEB 2086, may be useful therapeutic tools in the treatment of anaphylaxis-induced cardiac arrhythmias.

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