*Br. J. Pharmac.* (1985), **84,** 725–733

# Effects of the C5a anaphylatoxin and its relationship to cyclo-oxygenase metabolites in rabbit vascular strips

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**1** Strips of rabbit blood vessels were suspended *in vitro* and responses to complement peptides C3a and C5a were recorded isotonically.

**2** Human C3a (up to 1.5 μM) was inactive on rabbit vascular strips.

**3** Human C5a (2.9–59 nM) decreased spontaneous activity of the rabbit portal vein under resting baseline tension. The C5a relaxed strips of portal vein and pulmonary artery that were precontracted with noradrenaline (NA, 200 nM). On the portal vein, C5a-induced relaxation was preceded by a transient contractile phase which decreased with repeated applications of C5a. The magnitude of C5a-induced relaxation of both vessels increased with repeated stimulation by C5a. Maximal levels of relaxation for the third application of C5a at 59 nM averaged 44% and 17% of the NA-induced contraction plateau in portal vein and pulmonary artery, respectively.

**4** Strips of rabbit aorta responded minimally to C5a.

**5** Indomethacin (5.6 μM) significantly inhibited C5a-induced relaxation of the portal vein and pulmonary artery but had no effect on the early contractile response of the portal vein. Mepyramine (10 μM) failed to modify the C5a response from either vessel, but it reduced the contractile phase of the C5a response on the portal vein when applied in conjunction with indomethacin. The drug SKF 88046, an end organ antagonist of thromboxane (TX) $A_2$ and some contractile prostaglandins, reduced the contractile phase and increased relaxation of the portal vein to C5a but did not modify the response of the pulmonary artery.

**6** Radioimmunoassays for 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) and $TXB_2$ were performed on the fluid bathing rabbit isolated blood vessels. C5a promoted release of 6-keto-$PGF_{1\alpha}$ over the basal release rate in rabbit tissues. Only trace quantities of $TXB_2$ were produced by rabbit vessels exposed to C5a.

**7** It is concluded that the mechanical response of blood vessels to C5a is mainly determined by the type of cyclo-oxygenase products released and by the sensitivity of each blood vessel to these active lipids. Tissue histamine release is also responsible for a component of the response of rabbit portal vein to C5a. The relaxant effect of C5a on rabbit blood vessels may be a phenomenon related to the previously reported hypotensive action of classical anaphylatoxins *in vivo*.

## Introduction

When complement is activated in animal sera it becomes broncoconstrictive and vasoactive (see Bordet, 1913; Hicks & Sackeyfio, 1972). Complex cardiovascular responses to a peptide purified from complement-activated hog serum were reported in the guinea-pig (Bodamer & Vogt, 1967) and in the dog (Pavek *et al.*, 1979). That this peptide is the 'classical anaphylatoxin' $C5a_{des\,Arg}$ is now well established (Gerard & Hugli, 1981). A serum carboxypeptidase removes the C-terminal arginine residue from anaphylatoxins C3a, C4a and C5a when generated *in vivo*; but only the C5a molecule retains significant activity in the des-Arg form.

Porcine $C5a_{des\,Arg}$ is a hypotensive peptide both in the guinea-pig and in the dog. A reversible hypotensive phase followed by a tachyphylactic, catecholamine-dependent hypertensive phase was observed in the guinea-pig after intravenous injection of classical

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anaphylatoxin (Bodamer & Vogt, 1967; Hicks & Sackeyfio, 1972). In the dog, $C5a_{des\,Arg}$ reduced systemic arterial blood pressure but caused pulmonary hypertension (Pavek *et al.*, 1979). Indomethacin prevented the $C5a_{des\,Arg}$-dependent hypotension in the dog, suggesting that cyclo-oxygenase products mediate this cardiovascular effect of the anaphylatoxin.

The perfusion pressure of the guinea-pig lung was variably increased by $C5a_{des\,Arg}$ (Pavek *et al.*, 1979). Regal (1982) and Marceau & Hugli, (1984) have described a vasoconstrictor effect of C5a on guinea-pig isolated blood vessels. Vasoconstriction was apparently mediated by a combined effect of histamine and cyclo-oxygenase products. Furthermore, histamine and prostaglandins, but no slow reacting substance of anaphylaxis (SRS-A) activity, were detected by bioassay in the effluent of C5a-perfused lung tissue (Pavek *et al.*, 1979).

The present investigation was undertaken to characterize further the vascular effects of purified anaphylatoxin C5a in its fully active form, and eventually, describe an *in vitro* model for C5a-induced vascular relaxation related to its hypotensive effect *in vivo*.

## Methods

### *Isolated blood vessels*

New Zealand white rabbits (1.5–3 kg) were killed by stunning and exsanguination. Segments of blood vessels were removed, prepared and cut helically as described by Marceau & Hugli (1984). Three adjacent strips were generally obtained from each vessel segment and each strip was treated randomly with one of the three concentrations of C5a. The vascular strips were at least 8 mm long and 2 mm wide and were suspended in 2.5 ml organ bath containing oxygenated (95% $O_2$ and 5% $CO_2$) and warmed (37°C) Krebs solution. The composition of Krebs solution and the procedure for recording isotonic contractions were described previously (Marceau & Hugli, 1984).

### *Protocols*

The vascular strips were equilibrated for 45 min under a resting tension of 0.5 g. Most of the observations were performed on portal vein and pulmonary artery strips constricted with noradrenaline (NA). A moderate concentration ($ED_{30}$–$ED_{40}$) of NA (200 nM) was used to generate tone, so that vasodilator effects could be recorded, in a similar manner to Gagnon *et al.* (1978) and Förstermann *et al.* (1984). After NA application, a contraction plateau was reached and C5a was then applied. Changes elicited by C5a were expressed as either a positive (e.g. further enhancement of contraction) or negative (e.g. a relaxation) percentage of the NA-induced contraction. Typically, C5a produced a brief contraction followed by a relaxation of the rabbit portal vein and a relaxation of the pulmonary artery without a contraction phase. After the effect of C5a had worn off, tissues were washed and allowed to recover for 45 min, after which the procedure was repeated. Each tissue was exposed initially to NA, and then to NA and C5a for three successive applications as a means of assessing changes in the C5a effect during *in vitro* incubation. The first and third exposure to C5a typically occurred 1.5 and 4 h respectively from the start of the *in vitro* incubation.

In experiments with inhibitory drugs, the tissues were exposed to a constant concentration of the drug throughout the experiment.

### *Radioimmunoassays (RIA)*

To monitor the influence of C5a on arachidonate metabolism in vascular strips the bathing Krebs fluid was sampled at various times and submitted to a RIA for 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) and thromboxane $B_2$ ($TXB_2$). The $^3$H-RIA kits for these two compounds were purchased from New England Nuclear, Boston, MA. The samples (100 µl) were usually applied directly without extraction. Ether-extracted samples reconstituted in saline yielded quantitatively similar results to unextracted samples, suggesting that the immunoreactive substances were indeed lipids. Krebs solution was added to the samples of 6-keto-$PGF_{1\alpha}$ and $TXB_2$ used to construct standard curves, as suggested by the manufacturer of the kits, in order to compensate for solvent effects on the assays. Major crossreacting prostaglandins were $PGE_2$ (2.5%) for the 6-keto-$PGF_{1\alpha}$ assay and $PGD_2$ (2.0%) for the $TXB_2$ assay. Results are expressed in nM concentrations in the original bathing fluid.

### *Drugs*

(−)-Noradrenaline hydrochloride, mepyramine maleate (pyrilamine), histamine phosphate and indomethacin were purchased from Sigma (St Louis, MO.). SKF 88046 N, N′-bis[7-(3-chlorobenzeneaminosulphonyl)-1, 2, 3, 4-tetrahydroisoquinolyl]disulphonylimide, a competitive antagonist of bronchoconstrictor prostaglandins including $TXA_2$, $PGF_{2\alpha}$ and $PGD_2$ (Weichman *et al.*, 1984a,b), was a generous gift of Smith, Kline and French, Philadelphia, PA, U.S.A. Solutions of the drugs (0.1–1 mg $ml^{-1}$) were made in 0.9% saline and small volumes (1–20 µl) were injected into the tissue bath at the appropriate times. Indomethacin was dissolved in 1 M $NaHCO_3$ and SKF 88046 in 0.1 M $Na_2CO_3$.

*Anaphylatoxin C5a preparation*

Human anaphylatoxin C5a was used throughout the present studies and was prepared according to Hugli *et al.* (1981). C5a was homogeneous material as judged by cellulose acetate electrophoresis. Some experiments were performed using material obtained from the trailing end of the C5a peak eluted from CM Sephadex, as described by Fernandez & Hugli (1976). In the latter case, the C5a concentration was determined by bioassay (guinea-pig ileum) and this material produced the same response as homogeneous C5a on vascular strips prepared from guinea-pigs or rabbits. Homogeneous human C3a was also used in some experiments (Hugli *et al.*, 1981).

## Results

*Effect of C5a on rabbit isolated blood vessels*

Human C5a (up to 59 nM) exhibited minimal effects on resting rabbit blood vessels as measured by contraction. No contractile response to C5a was detected on the thoracic aorta or the pulmonary artery, tissues that exhibit neither resting tone nor spontaneous activity. However, the spontaneous activity of the portal vein was decreased by C5a (Figure 1a) and this response could be elicited repeatedly on the same strip. Relaxation of the portal vein was sometimes preceded by a small contraction. If the portal vein was first constricted with NA (200 nM), it generally exhibited a biphasic response to C5a at 2.9, 15 or 59 nM levels (Figure 1b, statistical analysis in Figure 2). A C5a-induced contraction exceeding the NA plateau was initially apparent and this effect was not strictly dose-dependent (Figure 2). The contractile effect was tachyphylactic since it decreased with subsequent applications of C5a. This contractile phase was highly variable and lacking in some animals. The second phase of the response was a relaxation of the tissue. Relaxation developed more slowly, was reversible, dose-dependent, and increased considerably in magnitude with subsequent applications of the same concentration of C5a (e.g. it doubled approximately from the first to the third application at 2.9 nM, see Figure 2). Maximal relaxation obtained on the portal vein averaged 44% after the third application of C5a using the highest concentration of 59 mM.

The response of NA-constricted pulmonary artery to C5a was generally a monophasic reversible relaxation (Figure 1c, Figure 2). Relative to the NA plateau, relaxation of the pulmonary artery was less marked than that of the portal vein; however, it was still dose-dependent and increased with subsequent applications of C5a. The rabbit thoracic aorta responded minimally to C5a (Figure 2).

In contrast to the C3a effect on guinea-pig blood vessels (Marceau & Hugli, 1984), human C3a (up to 1.5 μM) was unable to contract or relax rabbit blood vessels, either resting or constricted with NA.

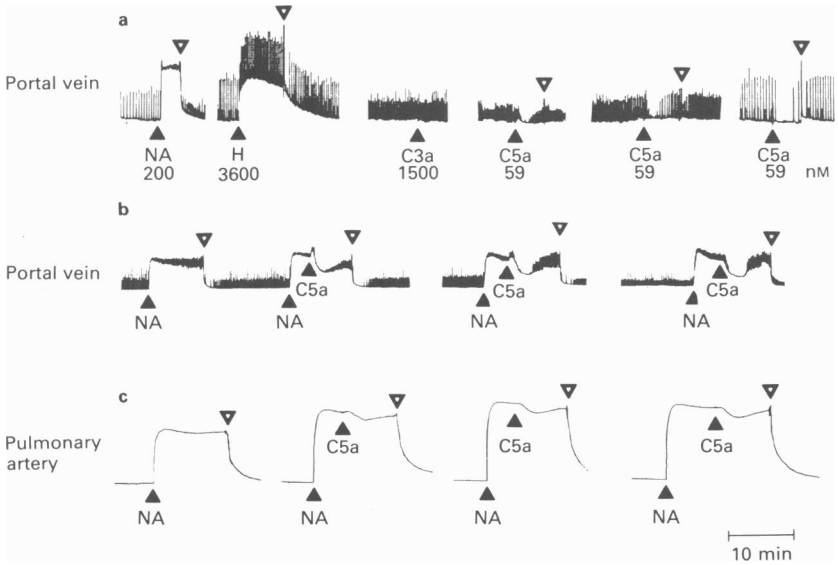


**Figure 1** (a) Effect of noradrenaline (NA), histamine (H), human C3a and C5a on the resting rabbit isolated portal vein. (b) Effect of C5a (59 nM) on the NA-constricted rabbit poral vein. (c) Effect of C5a (59 nM) on the NA-constricted rabbit pulmonary artery. Each vascular strip was exposed three times to C5a. Abscissa scale: time; ordinate scale: isotonic contraction. Closed symbols refer to the application of agents and open symbols to washout of stimulants.

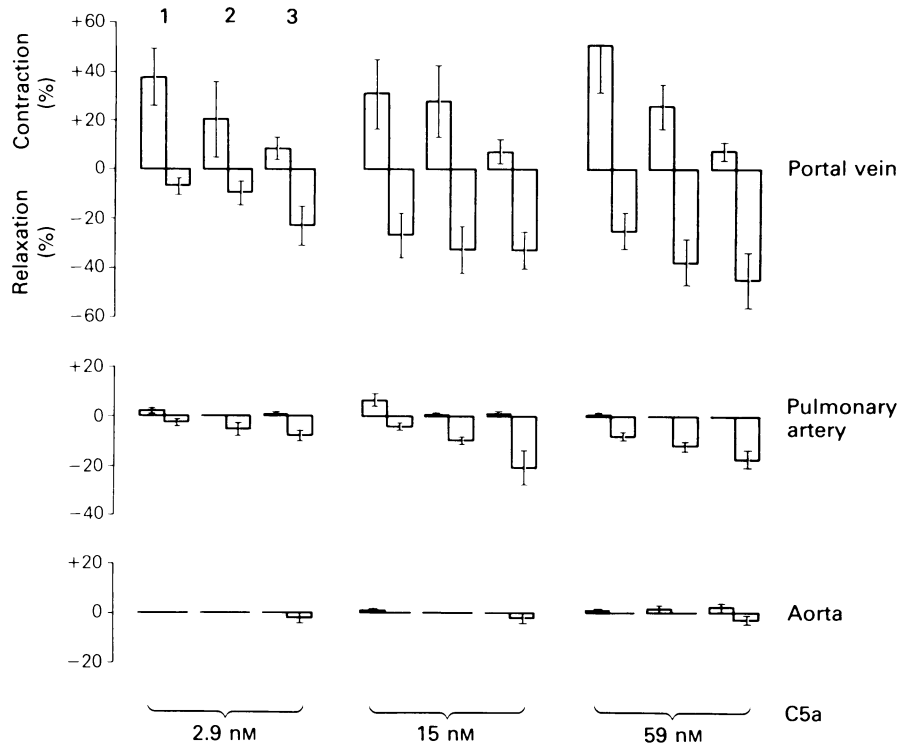


**Figure 2** Concentration-effect relationship of human C5a on rabbit blood vessels. Results are expressed as a positive (early contractile phase) and negative (late relaxation) percent of noradrenaline (NA)-induced plateau. Results of three consecutive applications of C5a on individual tissue strips are shown. Vertical bars indicate standard errors of 3 (aorta) or 8 determinations (portal vein and pulmonary artery).

*Effect of mepyramine, indomethacin and SKF 88046 on rabbit blood vessels challenged with C5a*

Continuous exposure to mepyramine (10 μM), a histamine $H_1$-receptor antagonist, failed to alter significantly the C5a response on either the portal vein (Figure 3) or the pulmonary artery (Figure 4). The cyclo-oxygenase inhibitor indomethacin, at 5.6 μM, either abolished or significantly decreased the relaxation phase induced by C5a on these tissues. An exception was the contractile phase observed on the portal vein after the first C5a application: this initial response was not significantly inhibited by indomethacin. A combination of mepyramine and indomethacin inhibited both phases of the C5a effect on the portal vein and this suggests that histamine and cyclo-oxygenase products account for all or most of the vasomotor action of C5a in this tissue. The magnitude of the contraction and quality of the plateau contraction induced by NA in the presence of mepyramine, indomethacin, or the combination of both drugs, were similar to the controls suggesting that drug-induced modification of C5a effects were specific and not due to tissue toxicity.

SKF 88046 was used at a concentration of 5.1 μM on rabbit isolated blood vessels, a concentration that markedly reduced or abolished the contractile effect of $PGF_{2\alpha}$ (up to 20 μM) on either the portal vein or the pulmonary artery. Continuous exposure to SKF 88046 did not modify significantly the pulmonary artery responses to C5a (2.9 or 59 nM) at any of three applications of the peptide. On the portal vein, SKF 88046 modified the response so that the first exposure to C5a became similar to the third response of the control tissues. That is, the early contractile phase was reduced and the late relaxation was increased after the first exposure. These trends were statistically significant at the 2.9 nM level of C5a (contractile phase reduced to 7.1 ± 3.2%, $P < 0.05$ and relaxation increased to 24.1 ± 4.7%, $P < 0.02$, $n = 5$).

*Radioimmunoassay of cyclo-oxygenase products*

In order to monitor the effects of C5a on arachidonate metabolism, vascular strips were exposed to C5a and aliquots of the bathing fluid were taken for analysis of 6-keto-$PGF_{1\alpha}$ and $TXB_2$. Förstermann *et al.* (1984) examined patterns of prostaglandin release from rab-

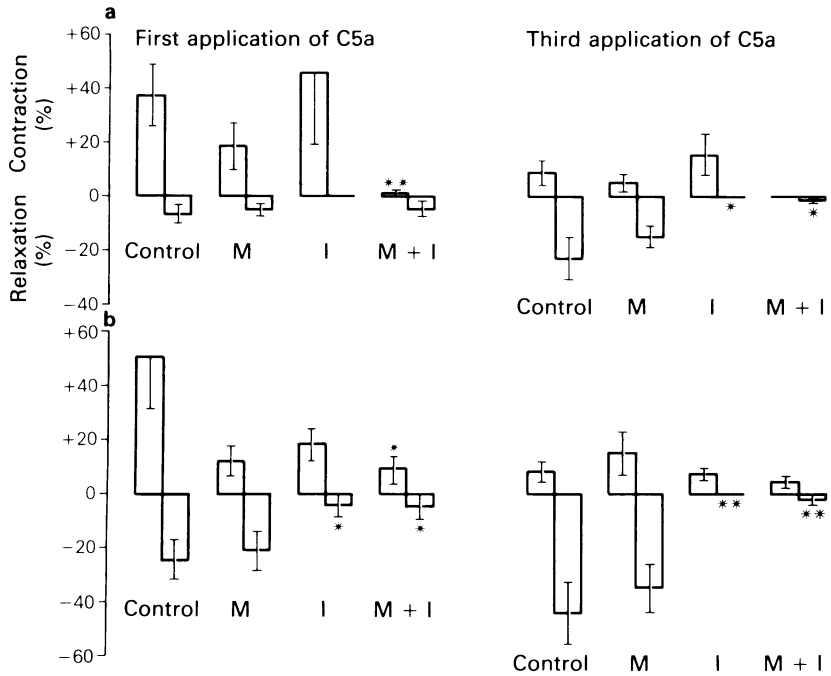


**Figure 3** Effect of mepyramine (M, 10 μM, $n = 7$), indomethacin (I, 5.6 μM, $n = 4$) or a combination of both (M + I, $n = 6$) on C5a-induced responses of the rabbit isolated portal vein. (a) Portal veins challenged with 2.9 nM C5a. (b) Portal veins challenged with 59 nM C5a. Results expressed as in Figure 2. Each phase of the response has been compared to the controls ($n = 8$) using Student's $t$ test and levels of statistical significance of difference with respect to controls are indicated by $*P < 0.05$ and $**P < 0.01$.

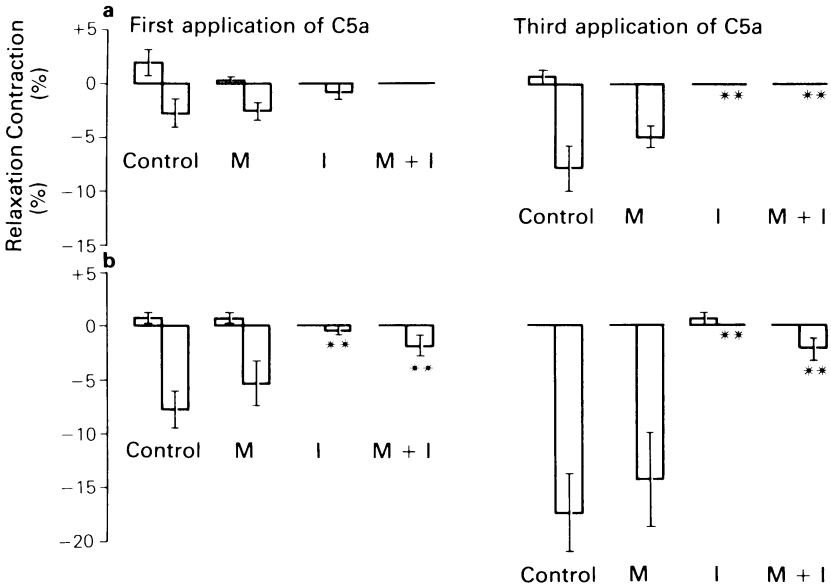


**Figure 4** Effect of mepyramine, indomethacin or a combination of both on C5a-induced responses of the rabbit isolated pulmonary artery. Concentration of drugs, abbreviations and group sizes are the same as in Figure 3. (a) Pulmonary arteries challenged with 2.9 nM C5a. (b) Pulmonary arteries challenged with 59 nM C5a. Each phase of the response has been compared to controls using Student's $t$ test ($*P < 0.05$ and $**P < 0.01$).

**Table 1** Characteristic responsiveness to C5a of vascular strips used in the analysis of cyclo-oxygenase products

| | *Tissues constricted with NA* | | *Resting tissues* | |
|---|---|---|---|---|
| | *Portal vein* | *Pulmonary artery* | *Portal vein* | *Pulmonary artery* |
| | *Mechanical response*[a] *Contraction/Relaxation* | | *Mechanical response* | |
| Effect of C5a (59 nM) | | | | |
| 1st application | +33/−25 | ±0/−8 | b | Nil |
| 3rd application | + 9/−47 | ±0/−13 | b | Nil |
| Wet weight of tissue | 18.5 mg | 19.4 mg | 18.6 mg | 23.5 mg |

[a]Biological effect of C5a on noradrenaline (NA)-constricted tissues expressed as a positive (early contractile phase) and a negative (late relaxation) percentage of NA-induced plateau. Two tissues were used for NA-constricted preparations and one for the resting tissue experiment.
[b]Decrease in spontaneous activity observed.

bit blood vessels. It appears that prostacyclin ($PGI_2$) is the predominant cyclo-oxygenase product released, followed by $PGE_2$, and that the ratio between these different products is maintained both during basal release conditions and after stimulation (e.g. with exogenous arachidonic acid). The present analysis has been limited to decay products of two active and physiologically antagonistic cyclo-oxygenase products, $PGI_2$ and $TXA_2$. It can be seen from Table 1 that vascular strips excised from animals assigned to this study gave typical responses to C5a.

There is a measurable basal release of $PGI_2$ from both rabbit portal vein and pulmonary artery tissue as judged by 6-keto-$PGF_{1\alpha}$ concentrations in the bathing fluid (Table 2). Ten minutes after washing fresh tissue, 6-keto-$PGF_{1\alpha}$ increased from undetectable levels (<0.27 nM) to 1.85–2.11 nM from portal vein and 0.67–1.03 nM from the pulmonary artery. Addition of NA ($2 \times 10^{-7}$ M) caused a moderate increase of 6-keto-$PGF_{1\alpha}$ concentrations in the bathing fluid at approxitely 3 min after application. Vasoconstrictors such as NA and angiotensin II are known to be moderate stimulators of cyclo-oxygenase products from vascular strips (Förstermann *et al.*, 1984). The tissues were then challenged with C5a (59 nM) and 6-keto-$PGF_{1\alpha}$ levels were measured after development of

**Table 2** Concentration of 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) and thromboxane $B_2$ ($TXB_2$) in the bathing fluid of rabbit vascular strips challenged with C5a

| | *Tissues contracted with NA* | | | | *Resting tissues* | | | |
|---|---|---|---|---|---|---|---|---|
| | *Portal vein* | | *Pulmonary artery* | | *Portal vein* | | *Pulmonary artery* | |
| | 6-keto-$PGF_{1\alpha}$ | $TXB_2$ | 6-keto-$PGF_{1\alpha}$ | $TXB_2$ | 6-keto-$PGF_{1\alpha}$ | $TXB_2$ | 6-keto-$PGF_{1\alpha}$ | $TXB_2$ |
| *First application* | | | | | | | | |
| Krebs after washing | <0.27[a] | <0.14 | <0.27 | <0.14 | — | — | — | — |
| Krebs, 10 min | | | | | | | | |
| after washing | 1.85 | 0.16 | 1.03 | <0.14 | 2.11 | <0.14 | 0.67 | <0.14 |
| + NA | 2.92 | <0.14 | 1.19 | <0.14 | — | — | — | — |
| + C5a | — | — | — | — | 4.05 | 0.23 | 1.11 | <0.14 |
| + NA + C5a | 6.10 | 0.22 | 1.72 | 0.24 | — | — | — | — |
| *Third application* | | | | | | | | |
| Krebs, 10 min | | | | | | | | |
| after washing | 0.42 | <0.14 | 0.38 | <0.14 | 0.57 | <0.14 | 0.67 | <0.14 |
| + NA | 0.73 | <0.14 | 0.69 | <0.14 | — | — | — | — |
| + C5a | — | — | — | — | 1.84 | <0.14 | 1.32 | <0.14 |
| + NA + C5a | 2.47 | <0.14 | 1.04 | <0.14 | — | — | — | — |

[a]Nanomolar concentrations measured in the Krebs fluid bathing vascular strips characterized in Table 1. Bath volume 2.5 ml. Tissues were washed, allowed to rest 10 min and either challenged sequentially with noradrenaline (NA, 200 nM) and C5a (59 nM) or with C5a alone. Concentrations reflect cumulative release of mediators. Samples were collected 3 min after a NA challenge and 5–6 min after a C5a challenge.

the biological response. Total levels of 6-keto-$PGF_{1\alpha}$ increased considerably (up to 6.10 nM on the portal vein) after C5a stimulation. C5a also induced a measurable increase in the $TXB_2$ concentration in most cases (up to 0.24 nM). Changes in $TXB_2$ levels were considerably less than those for 6-keto-$PGF_{1\alpha}$ and basal release levels of $TXB_2$ were usually below the limits of detection (< 0.14 nM).

Prostanoid output was generally less pronounced from pulmonary artery strips than from portal veins on a wet weight basis. Injection of C5a on non-constricted (i.e. NA-free) vascular strips also increased the concentration of 6-keto-$PGF_{1\alpha}$ and $TXB_2$ in the supernatant; this suggests that NA is not involved in C5a-stimulated release of $PGI_2$ and $TXA_2$.

Both basal and stimulated release of 6-keto-$PGF_{1\alpha}$ and $TXB_2$ generally decreased in rate after 3.5 h of incubation; this period included a second challenge by C5a. Results after a third exposure to NA and C5a are given in Table 2.

Indomethacin (5.6 μM) inhibited by 83% or more the release of immunoreactive 6-keto-$PGF_{1\alpha}$ from freshly isolated rabbit portal veins; $TXB_2$ release was undetectable under these conditions.

## Discussion

The present data suggest that the active fragment C5a of the complement system can stimulate vasomotor mechanisms. C5a elicited contractile responses in certain isolated blood vessels from the guinea-pig (Regal, 1982; Marceau & Hugli, 1984); conversely a relaxant response was observed on rabbit vascular strips. Despite opposing mechanical effects of C5a on vessels excised from these two species, similarities in response were observed for tissues taken from both animals. The C5a response in both species involved primarily cyclo-oxygenase products with a minor contribution by histamine. In the rabbit portal vein, histamine partially mediated the initial transient contractile phase elicited by C5a. Organ responsiveness was the same in both rabbits and guinea-pigs; the portal vein was consistently the most responsive of the blood vessels sampled while the pulmonary artery and the aorta were less reactive. One surprising observation was the lack of an effect of human C3a on rabbit vascular strips because guinea-pig blood vessels have been shown previously to be contracted by human C3a (Marceau & Hugli, 1984). We cannot rule out species specificity as the reason for insensitivity of the rabbit tissue, since human C3a may not recognize C3a receptors on rabbit cells.

The actual mechanical response of a given blood vessel to C5a is apparently a function of several variables; however, we believe that the response depends on a capacity of the tissue to form various prostanoids and on the sensitivity of that tissue to these lipid mediators. Strips of guinea-pig portal vein and pulmonary artery released large amounts of $TXA_2$ relative to $PGI_2$ in their bathing fluid, especially after C5a stimulation (Marceau & Hugli, unpublished results). This high ratio of $TXA_2$ to $PGI_2$, in combination with histamine release, could account for the contractile effect of C5a in this species. Nevertheless, $PGI_2$ was released by C5a from guinea-pig vascular tissues and this release might be significant in certain vascular beds because the classical anaphylatoxin is known to be hypotensive in the anaesthetized guinea-pig (Bodamer & Vogt, 1967). The rabbit isolated vessels produced vasoconstrictor prostanoids such as $TXA_2$ (present results) and $PGF_{2\alpha}$ (Förstermann *et al.*, 1984). However, indomethacin-sensitive relaxation is the predominant response of rabbit vascular tissue to C5a, especially on repeated stimulation.

Previous results might help to explain the relative amplitude of C5a-induced relaxation in the three sampled blood vessels. The NA-constricted rabbit aorta failed to relax when exposed to $PGE_2$ and $PGI_2$, whereas the pulmonary artery was moderately relaxed by $PGE_2$ while $PGI_2$ had a minimal effect (Förstermann *et al.*, 1984). The coeliac artery was very sensitive to the relaxant effect of $PGI_2$ and $PGE_2$ in the same study. The rabbit portal vein appears especially sensitive to vasodilator prostaglandins, as demonstrated for prostaglandins of the E series (Kitamura *et al.*, 1976). These findings suggest that C5a responses are primarily determined by the sensitivity of each blood vessel to the individual prostanoids released.

Multiple mediator interactions probably account for the vascular effect of C5a on rabbit tissue. The early contractile event elicited by C5a on the portal vein was inhibited by a combination of mepyramine and indomethacin; this suggests a role for histamine and vasoconstrictor prostanoid(s). The amplitude of the relaxation induced by C5a increased following subsequent applications of C5a on both the portal vein and the pulmonary artery, despite the fact that cyclo-oxygenase product output decreased, as measured by RIA. The increased vasodilator effect recorded on subsequent applications of C5a was fully inhibited by indomethacin. The mechanism of this sensitization remains to be elucidated but may correlate with a decreased influence of vasoconstrictive mediators on target tissue. This interpretation is supported by the effect of SKF 88046 on the portal vein. This agent, a competitive antagonist of several constrictor prostanoids on guinea-pig tissues (Weichman *et al.*, 1984a,b), apparently inhibited the contractile phase and uncovered the full relaxation effect of C5a at its first application. However, SKF 88046 did not potentiate relaxation of the pulmonary artery and evidence derived from its utilization will be of limited value until

its activity against various prostanoids is better elucidated in vascular systems.

C5a stimulates the release of low-molecular weight agents, especially histamine and various arachidonic acid metabolites. These secondary mediators, in variable proportions, seem to account entirely for the action of C5a on various smooth muscle preparations from the guinea-pig. The influence of C5a has been characterized on vascular tissues (Regal 1982; Marceau & Hugli, 1984), tracheal and pulmonary strips (Regal & Pickering 1981; Stimler *et al.*, 1981; 1982) and the ileum (Cochrane & Müller-Eberhard, 1968). The present data on rabbit blood vessels further support the lack of a direct effect of C5a on smooth muscle. The cell types responsible for production of cyclo-oxygenase products remain to be identified although endothelial cells are the major cellular source of $PGI_2$ and are also a significant source of $TXA_2$ in some species (Higgs & Moncada, 1983). Therefore, it will be interesting to investigate whether these cells possess receptors for C5a or are indirect targets for mediators released from tissues by C5a.

It is tempting to speculate that intravascular activation of complement can lead to prostaglandin-mediated vasodilatation in some organs and contribute to inflammatory changes. Vasodilator prostaglandins, such as $PGE_2$ and $PGI_2$, are suspected of increasing blood flow in inflamed areas and contributing to exudate formation in conjunction with other factors active on vascular permeability (Wedmore & Williams, 1981; Williams & Morley, 1973). In addition to the present data, Rampart *et al.* (1983) showed that tryptic fragment of human C5, behaving like C5a on gel filtration, could release $PGI_2$ from rabbit mesentery pieces. Thus, C5a could be an important link between complement activation and the inflammatory vascular event.

This is publication number IMM 3588 from Scripps Clinic and Research Foundation, La Jolla, California. This work is supported by NIH Grants HL25658, HL16411 and AI17354. F.M. has been a Fellow of the Medical Research Council of Canada and the Arthritis Society of Canada. The authors wish to thank Dr Barry M. Weichman of Smith, Kline and French Laboratories for the gift of SKF 88046. We acknowledge the secretarial assistance of Ms Ellye Lukaschewsky in the preparation of this manuscript.

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(*Received August 23, 1984.*
*Revised October 8, 1984.*
*Accepted October 15, 1984.*)