The present study was performed to investigate the early signalling events responsible for eosinophil activation in response to platelet-activating factor (PAF), C5a, and leukotriene B4 (LTB4). We evaluated the effect of pertussis toxin (PTX) on eosinophil aggregation in vitro and cutaneous eosinophil recruitment in vivo. Further studies using the protein kinase inhibitors Ro 31-8220 and staurosporine were performed in vitro to assess in more detail the early signalling events induced by these three mediators. Our results show that C5a and LTB4 signal predominantly or exclusively through a PTX-sensitive G protein that is negatively modulated by protein kinase C, possibly at the level of phospholipase C-β. In contrast, PAF activates eosinophils independent of Gi by a mechanism that is abolished by Ro 31-8220, a selective protein kinase C inhibitor. In addition, these results show for the first time that a receptor-operated event on the eosinophil is essential for chemotactant-induced eosinophil recruitment in vivo.

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MATERIALS AND METHODS

Purification of guinea pig peritoneal eosinophils. The method of eosinophil purification is described in detail elsewhere. Briefly, ex-breeder female guinea pigs (Harlan, Oxon; 700 to 800 g) were treated with undiluted horse serum (1 mL intraperitoneally) every other day for 2 to 3 weeks and the cells were harvested by peritoneal lavage with heparinized saline (10 IU/mL) 2 days after the last injection. The cells obtained were layered onto a discontinuous Percoll-Hanks’ balanced salt solution (HBSS; calcium- and magnesium-free) gradient followed by centrifugation (1,500g for 25 minutes at 20°C). Eosinophils (>95% pure, >98% viable) were collected from the 1.090/1.095 and 1.095/1.100 g/mL density interfaces. The cells were then washed twice in Dulbecco’s phosphate-buffered saline.
Eosinophil aggregation. Aggregation experiments were performed as previously described. Briefly, guinea pig eosinophils were resuspended (5 x 10^7 cells/mL) in PBS, CaCl₂ and MgCl₂ (final concentrations, 1.0 mMol/L and 0.7 mMol/L, respectively) were added, and the cells were kept on ice. Five minutes before use, the cells were warmed to 37°C and aliquots (300 µL) were dispensed into siliconized cuvettes that were placed into a dual-channel platelet aggregometer (Chronolog 440 VS) linked to a dual pen recorder (Chronolog 707). The cells were incubated for at least 5 minutes at 37°C with continuous stirring at 700 rpm before stimulation with the indicated agonist. The reference cuvette contained buffer alone. Responses were allowed to develop for at least 3 minutes and were measured at the peak of aggregation. Results are expressed as the percentage of maximal aggregation induced by 10⁻⁷ mol/L phorbol myristate acetate (PMA). Eosinophils were incubated with the various pharmacologic agents (Ro 31-8220, staurosporine, and H89) for 2 to 3 minutes and then stimulated with PAF (10⁻¹⁰ mol/L to 10⁻⁷ mol/L), C5a (10⁻⁸ mol/L to 10⁻⁷ mol/L), or LTB₄ (10⁻¹⁰ mol/L to 10⁻⁷ mol/L).

Measurement of intracellular calcium. Purified guinea pig eosinophils (5 x 10⁶ cells/mL in PBS with 0.25% bovine serum albumin (BSA)) were loaded with fura-2-acetoxymethyl ester (2.5 µmol/L, 30 minutes at 37°C). After two washes, eosinophils were resuspended at 10⁷ cells/mL in PBS buffer containing 10 mMol/L HEPES, 0.25% BSA, and 1 mMol/L calcium and stored on ice. Ten minutes before their use, eosinophils were warmed to 37°C and 300-µL aliquots were dispensed into quartz cuvettes. Changes in fluorescence were monitored at 37°C using a spectrometer (LS50; Perkin-Elmer Corp, Beaconsfield, Bucks, UK) at excitation wavelengths 340 nm and 380 nm and emission wavelength 510 nm. [Ca²⁺]i levels were calculated using the ratio of the two fluorescence readings and a kd for Ca²⁺ binding at 37°C of 224 nmol/L. As with the aggregation experiments, eosinophils were incubated with Ro 31-8220 and staurosporine for 3 minutes and then activated with PAF, C5a, or LTB₄.

Radiolabeling of eosinophils and recruitment in vivo. Purified eosinophils were radiolabeled with ¹¹¹In as previously described. The ¹¹¹In-radiolabeled cells were then incubated at 37°C with PTX or vehicle for 2.5 hours (see below), washed twice, and injected intravenously (2.5 x 10⁷ cells/animal) together with 125I-human serum albumin (250-µCi) into recipient guinea pigs (350 to 400 g) sedated with Hypnorm (0.15 mL intramuscularly). After 5 minutes, duplicate intradermal injections of inflammatory stimuli (PAF at 10⁻¹² to 10⁻⁹ mol/site, LTB₄ at 10⁻¹² to 10⁻¹⁰ mol/site, and C5a at 10⁻¹₂ and 10⁻¹¹ mol/site) were administered in 0.1-mL volumes into the shaved dorsal skin following a randomized injection plan. ¹¹¹In-labeled eosinophil accumulation and ¹²⁵I-HSA extravasation were assessed 1 hour after intradermal injection of mediators. At this time, a blood sample was obtained by cardiac puncture, the animals were killed with an overdose of sodium pentobarbitone, the dorsal skin was removed and cleaned free of excess blood, and the sites were punched out with a 17-mm punch. The samples were counted in an automatic 5-head gamma-counter (Canberra Packard Ltd, Pangbourne, Berks, UK) and the counts were cross-channel corrected for the two isotopes. Eosinophil numbers in the skin sites were expressed as the number of ¹¹¹In-eosinophils per skin site and ¹²⁵I-HSA extravasation was expressed as microliters of plasma.

PTX treatment. Purified eosinophils were diluted in PBS (5 x 10⁶ cells/mL in PBS with 0.25% BSA and 10 mMol/L HEPES) and incubated with PTX (1 µg/mL) at 37°C for 2.5 hours. An aliquot was separated and labeled with Fura-2 as described above and the cells were incubated for a further 30 minutes. The eosinophils were washed and diluted in PBS containing 0.25% BSA and 10 mMol/L HEPES (5 x 10⁶ cells/mL) for the aggregation experiments and 10⁶ cells/mL for calcium measurements). Magnesium (0.8 mMol/L, for the aggregation experiments only) and calcium (1.0 mMol/L were added back and the cells were kept on ice until use. The activation of eosinophils with PAF, C5a, and LTB₄ was performed on the same PTX-treated cell preparations. For the in vivo experiments, ¹¹¹In-labeled eosinophils were diluted in PBS (5 x 10⁶ cells/mL in PBS with 0.25% BSA and 10 mMol/L HEPES) and incubated with PTX (1 µg/mL) at 37°C for 2.5 hours. The cells were then washed twice and resuspended in a final volume of 5 x 10⁶ ¹¹¹In-eosinophils/mL.

Material. The following reagents were purchased from Sigma Chemical Co (Poole, UK): BSA, D-glucose, dimethyl sulfoxide (DMSO), PMA, PTX, and staurosporine. Horse serum, Dulbecco’s PBS (calcium- and magnesium-free, pH 7.4), and HBSS were from Life Technologies Ltd (Paisley, UK). Percoll was from Pharmacia (Milton Keynes, UK). C16 PAF was from Bachem (Saffron Walden, UK) and LTB₄ was from Cascade (Reading, UK). Human recombinant C5a (C5a) was a gift from Dr J. van Oostrum (Ciba Geigy, Summit, NJ). The protein kinase A inhibitor, H89, was purchased from Biomol (Nottingham, UK) and was dissolved in 50% ethanol. The protein kinase C inhibitor Ro 31-8220 was a gift from Dr D. Bradshaw (Roche, Welwyn Garden City, UK). Stauroporine and Ro 31-8220 were dissolved initially in DMSO and further diluted in appropriate assay buffer. None of the drug vehicles had any significant effect on aggregation or calcium responses induced by any of the agonists tested (data not shown).

Statistical analysis. Results were analyzed using analysis of variance (ANOVA) with the statistical program Instat (GraphPad Software V2.03). P values were assigned using Student-Newman-Keuls post-test. When only two groups were compared, the Student’s t-test was performed. Results were considered significant when P < .05. Data are presented as the mean ± SEM of n experiments.

RESULTS

Effects of PTX on eosinophil aggregation and intracellular calcium transients. Experiments were designed to assess the nature of the G-proteins that couple to the seven domain transmembrane spanning receptors for PAF, C5a, and LTB₄ by using the bacterial toxin, PTX. Eosinophils were pretreated for 2.5 hours with PTX (1 µg/mL) or its vehicle and agonist-induced aggregation and Ca²⁺ mobilization were subsequently assessed. PAF, C5a, and LTB₄ elicited the aggregation of guinea pig eosinophils in a concentration-dependent manner with approximate EC₅₀ values of 2 x 10⁻⁹ mol/L, 2 x 10⁻⁸ mol/L, and 3 x 10⁻⁹ mol/L, respectively (see Fig 4). Figures 1 and 2 show typical agonist-induced aggregation and intracellular calcium transient traces of control and PTX-treated cells. Whereas PAF-induced aggregation and Ca²⁺ mobilization were unaffected in PTX-treated eosinophils, the same responses evoked by LTB₄ were markedly reduced (75% to 90%) and those effected by C5a were abolished (Table 1).

Effects of PTX on the recruitment of ¹¹¹In-eosinophil into guinea pig skin. In agreement with previous reports, intradermal injection of LTB₄ (10⁻¹² to 10⁻¹⁰ mol/site), PAF (10⁻¹¹ to 10⁻⁹ mol/site), and C5a (10⁻¹² and 10⁻¹¹ mol/site) into the skin of guinea pigs induced a dose-dependent recruitment of ¹¹¹In-labeled eosinophils (Fig 3). Because the activation of seven transmembrane spanning G-protein-coupled receptors by inflammatory stimuli is believed to play a fundamental role in the recruitment of leukocytes to sites of inflammation in vivo, studies were performed to determine if the mechanism by which LTB₄, PAF, and C5a promote...
cutaneous eosinophilia and edema formation could be differentiated by PTX, as might be predicted from the in vitro results described above. As shown in Fig 3, the recruitment of $^{111}$In-eosinophils induced by C5a and LTB$_4$, was abolished by pretreatment of eosinophils with PTX before their intravenous injection into recipient animals. In contrast, $^{111}$In-eosinophil accumulation induced by PAF was inhibited by approximately 50% in the same animals under identical experimental conditions (Fig 3). The reduction in the recruitment of $^{111}$In-eosinophils into skin sites could not be attributed to a reduction in the ability of PTX-treated cells to circulate in recipient animals. Indeed, the percentage of control $^{111}$In-eosinophils circulating at 1 hour was 10.8% ± 3.2% (n = 5) and the corresponding value for PTX-treated $^{111}$In-eosinophils was 15.3% ± 4.9% (n = 5).

The extravasation of $^{125}$I-HSA in response to PAF, C5a, and LTB$_4$, was also measured in the same sites as $^{111}$In-eosinophil accumulation to confirm that animals that received PTX-treated cells responded normally to the intradermal stimuli. LTB$_4$ induced no significant edema formation over saline background (data not shown), whereas PAF- and C5a-induced edema responses were unaltered in animals that received PTX- or vehicle-treated $^{111}$In-eosinophils (eg, control animals, PAF at $10^{-10}$ mol/site, 61.7 ± 6.8 μL of plasma, and C5a at $10^{-11}$ mol/site, 51.1 ± 6.7 μL; animals receiving PTX-treated eosinophils, PAF, 61.8 ± 5.2 μL, and C5a, 54.5 ± 3.0 μL; n = 5).

Effect of the protein kinase inhibitors Ro 31-8220, staurosporine, and H89 on eosinophil aggregation. Given that the results obtained with PTX indicate that the receptors for PAF, C5a, and LTB$_4$ couple to distinct G-proteins in eosinophil membranes, further experiments were conducted to assess in more detail the early signalling events by which these agonists promote aggregation and Ca$^{2+}$ mobilization. Pretreatment of eosinophils with the PKC inhibitor, Ro 31-8220, at a concentration ($3 \times 10^{-6}$ mol/L) that abolished PMA-induced aggregation (data not shown) suppressed PAF-induced aggregation by between 90% and 100% depending on the agonist concentration used (Fig 4A) but had little effect on aggregation evoked by C5a except at the highest concentration examined, in which aggregation was potentiated (Fig 4B). Curiously, aggregation induced by $10^{-10}$ and $10^{-9}$ mol/L LTB$_4$ was markedly inhibited by Ro 31-8220 (Fig 5A). Because staurosporine is known to inhibit protein kinases in addition to PKC, it was hypothesised that the augmentation of the aggregation response might be due to an effect on PKA that generally exerts an inhibitory
DISTINCT EARLY SIGNALLING EVENTS IN EOSINOPHIL ACTIVATION

Table 1. Effect of PTX (1 µg/mL, 2.5 Hours at 37°C) on Eosinophil Aggregation Induced by PAF, C5a, and LTB4

<table>
<thead>
<tr>
<th>Aggregation (% maximal response induced by PMA)</th>
<th>Control</th>
<th>PTX</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAF (10^-8 mol/L)</td>
<td>16.3 ± 4.2</td>
<td>13.3 ± 5.2</td>
</tr>
<tr>
<td>C5a (10^-7 mol/L)</td>
<td>24.8 ± 2.5</td>
<td>0*</td>
</tr>
<tr>
<td>LTB4 (10^-8 mol/L)</td>
<td>20.5 ± 1.2</td>
<td>5.0 ± 2.0*</td>
</tr>
</tbody>
</table>

Values are the mean ± SEM of three to four experiments.
*P < .01 when compared with control values.

Effect in eosinophils (eg, Teixeira et al13). As shown in Fig 5B, the PKA inhibitor, H89, at a concentration (10^-5 mol/L) shown previously to inhibit PKA in intact neutrophils,21 significantly enhanced PAF-, C5a-, and LTB4-induced eosinophil aggregation.

Effect of the protein kinase inhibitors Ro 31-8220 and staurosporine on Ca2+ mobilization in eosinophils. The effect of Ro 31-8220 and staurosporine on the Ca2+ transient evoked by PAF, C5a, and LTB4 is shown in Table 2. Data are expressed as the area under the curve, which takes into account both the amplitude and the duration of the Ca2+ signal. Whereas PAF-induced Ca2+ mobilization was abolished in eosinophils pretreated with Ro 31-8220 (3 × 10^-5 mol/L), equivalent responses elicited by LTB4 and C5a were significantly enhanced. This effect was due predominantly to an increase in the duration rather than the peak height of the Ca2+ transient (see Fig 6 for a typical LTB4 response). Lower concentrations of C5a and LTB4 were affected similarly by pretreatment with Ro 31-8220 (data not shown). In...
Fig 6. Typical traces showing the effect of Ro 31-8220 and staurosporine on intracellular calcium transients induced by a maximally effective concentration of LTB₄. Eosinophils were labeled with fura-2-acetoxymethyl ester and washed and changes in fluorescence were monitored. The cells were pretreated for 3 minutes with vehicle, Ro 31-8220 (3 × 10⁻⁵ mol/L), or staurosporine (10⁻⁷ mol/L) before the addition of LTB₄ (10⁻⁸ mol/L, shown by the arrows). Results are expressed as the increase in intracellular calcium in nanomoles per liter as a function of time (in seconds).

contrast, pretreatment of eosinophils with staurosporine (10⁻⁷ mol/L) did not affect the Ca²⁺ transient evoked by PAF, whereas the same response elicited by C5a and LTB₄ was markedly enhanced. Again, this effect was due predominantly to an increase in the duration rather than the amplitude of the Ca²⁺ transient (see Fig 6 for a typical LTB₄ response).

DISCUSSION
In the present study the effects of PTX and inhibitors of PKC and PKA on PAF-, C5a-, and LTB₄-induced homotypic aggregation and changes in the cytosolic free Ca²⁺ concentration in guinea pig eosinophils were assessed. Collectively, the results presented herein strongly suggest that the early signalling events that ultimately promote aggregation and Ca²⁺ mobilization differ fundamentally between the three stimuli. Unequivocal evidence for this contention was obtained from studies in which eosinophils were pretreated with PTX. Thus, whereas C5a-induced aggregation and Ca²⁺-mobilization were abolished in PTX-treated cells, the same responses evoked by LTB₄ were inhibited by between 75% and 90% and those evoked by PAF were unaffected. In contrast, pretreatment of eosinophils with staurosporine (10⁻⁷ mol/L) did not affect the Ca²⁺ transient evoked by PAF, whereas the same response elicited by C5a and LTB₄ was markedly enhanced. Again, this effect was due predominantly to an increase in the duration rather than the amplitude of the Ca²⁺ transient (see Fig 6 for a typical LTB₄ response).
human eosinophils express a number of G-proteins that include Goα13, Goα16, Goαq11, and Goα24. Although it cannot be stated with certainty the type of G-protein involved in LTB4- and C5a-induced aggregation and Ca2+-mobilization, Goα is unlikely to represent the PTX substrate because it is believed to be coupled primarily, although not exclusively (see Padrell et al36), to the activation of voltage-sensitive Ca2+ channels.27 Because Goα is not present in guinea pig eosinophils35 a case can be made for Goα3/Goα4. In particular, both of these G-proteins are PTX substrates and couple to phospholipase (PLC)-β in a number of cells and tissues, which almost certainly include eosinophils (see below). The identity of the G-protein that promotes aggregation and Ca2+-mobilization in response to PAF is less clear. Although PTX failed to inhibit PAF-induced functional responses in eosinophils, the involvement of Goα was not initially excluded. It was reasoned that the PTX insensitivity might occur if Goα is predominantly either unaffected, inhibited, or enhanced (depending on the cells of the hematopoetic lineage,28,29 cannot be excluded. In likely target for PKC in this respect is PLC-αa which has been detected in guinea pig eosinophils,25 although it cannot be shown that the animals that received PTX-treated eosinophils is based on the suggestion that phosphorylation of PLC-αa is potentially inhibited by approximately 50% under identical experimental conditions. It is noteworthy that, in the same animals, edema responses induced by PAF and C5a were unaltered, showing that the animals that received PTX-treated eosinophils were responding normally to the exogenous intradermal stimulants. Collectively, our results provide convincing evidence that a receptor-operated event on the eosinophil is essential for eosinophil migration in vivo. These data are entirely consistent with what has been shown previously for neutrophils and lymphocytes.19,31 Furthermore, in vivo results suggest that the early signalling events that ultimately induce 111In-eosinophil accumulation in guinea pig skin also differ between PAF, C5a, and LTB4. The partial inhibition of PAF-induced 111In-eosinophil accumulation by PTX was contrary to what would be predicted from the in vitro results. However, PAF-induced 111In-eosinophil accumulation in guinea pig skin is partially dependent on the release of a 5-lipoxygenase product, probably LTB4,16 which would account for the PTX-inhibitable component of PAF-induced cutaneous eosinophilia.

Further evidence for a difference in signalling between PAF, C5a, and LTB4 was obtained from the finding that PAF-induced eosinophil aggregation and Ca2+-mobilization were abolished by the PKC inhibitor, Ro 31-8220, whereas the duration of the Ca2+ transient evoked by C5a and LTB4 was prolonged, and eosinophil homotypic aggregation was either unaffected, inhibited, or enhanced (depending on the concentration of stimulus used; see Table 3). Thus, it is apparent from our data that PKC is an essential early signalling event after PAF activation of guinea pig eosinophil aggregation and Ca2+-mobilization. The results with C5a and LTB4 are consistent with data recently published by Perkins et al,32 who reported that Ro 31-8220 potentiated LTB4-induced hydrogen peroxide generation, the duration of the Ca2+ transient, and the magnitude of the inositol (1,4,5)-trisphosphate [Ins(1,4,5)P3] signal in guinea pig eosinophils. Taken together, these data may be explained if PKC, when activated in response to C5a and LTB4, exerts a negative feedback influence on one or more effectors of Ca2+ mobilization and homotypic aggregation that is relieved by Ro 31-8220. Although there are several proteins that could fulfill this role, including Ins(1,4,5)P3 5-phosphatase,33,34 the most likely target for PKC in this respect is PLC-β.35 Indeed, phosphorylation of PLC-β is regarded as a ubiquitous mechanism of regulation that results in a decrease in phosphoinositide hydrolysis and coincident desensitization of PLC-β-coupled receptors.35

The observation that the aforementioned effects of Ro 31-8220 were dependent on the activating stimulus is both intriguing and perplexing. This is confounded further by the finding that PAF and LTB4 promote the generation of Ins(1,4,5)P3 in guinea pig eosinophils,32,36 indicating that stimulation of PLC is an early signalling event that follows the ligation of both receptors. Based on available data, a number of possibilities could explain the divergent effects of Ro 31-8220 on agonist-induced Ca2+-mobilization and aggregation that are not mutually exclusive. First, PAF receptors may be coupled to an isoform of PLC that is not negatively regulated by PKC. Although the complement of PLC isoenzymes in eosinophils is unknown, a possible candidate could include PLC-ε, which has been identified as a major G-protein–regulated enzyme.37 A second explanation is based on the suggestion that phosphorylation of PLC-β by PKC decreases inositol phospholipid hydrolysis, probably by altering the efficiency by which it couples to the appropriate G-protein rather than by inhibiting enzyme activity per se.35 It is possible that phosphorylation of PLC-β by PKC does not affect equally the coupling efficiency of all G-proteins.32,33 According to this paradigm, Gi3/2 (or Giα2) might
not interact optimally with phosphorylated PLC-β under conditions in which the PTX-insensitive G-protein(s) that mediates the effects of PAF in eosinophils is not affected. Third, the potential multiplicity of βγ subunits liberated from activated G-proteins that are associated with PAF, LTB4, and C5a receptors may exert opposing effects on the activity of PLC-β and/or other effectors of Ca2+-mobilization and aggregation. Finally, PKC may differentially mediate desensitization of PAF, LTB4, and C5a receptors. However, this latter possibility is less likely, because activation of PKC with phorbol esters is accompanied by a diminished functional response of eosinophils to both PAF and LTB4.

The inability of PTX to abolish LTB4-induced Ca2+-mobilization and aggregation suggests that LTβ receptors might couple to more than a single G-protein. This contention is entirely consistent with the promiscuous coupling of other receptors to multiple G-proteins (eg, Asano et al.39 and Rooney et al.40) and is strengthened by the results obtained with Ro 31-8220, which both inhibited and potentiated aggregation depending on the concentration of LTB4 tested. It is noteworthy that previous studies have unequivocally identified two populations of LTB4 receptors on guinea pig eosinophils,32,42 which raises the possibility that they can independently couple to PTX-sensitive and -insensitive G-proteins. However, our results in vivo suggest that a PTX-sensitive pathway is most important for LTB4-induced eosinophil recruitment in guinea pig skin.

In contrast to the results obtained with Ro 31-8220, whose effects on eosinophil activation were agonist-dependent (see above), the nonselective protein kinase inhibitor, staurosporine,29 invariably augmented PAF-, C5a-, and LTB4-induced Ca2+-mobilization and aggregation. Although the mechanism of action of staurosporine was not formally investigated in this study, the finding that qualitatively identical data were obtained when H-89 was used suggests that staurosporine may be acting primarily as an inhibitor of PKA. Whereas Ro 31-8220 abolished PMA-induced aggregation, staurosporine blocked the responses by only 75%. Thus, it is possible that an effect of staurosporine on PKA may counteract the partial PKC inhibition and exert a negative tonic influence on Ca2+-homeostasis and the aggregation response in guinea pig eosinophils. Indeed, a similar conclusion was reported for PAF-induced platelet aggregation.43

In conclusion, the data presented herein suggest that the early signalling events that affect Ca2+-mobilization, homotypic aggregation, and in vivo recruitment of guinea pig eosinophils are dependent on the nature of the activating stimulus. Specifically, C5a and LTB4 signal predominantly or exclusively through a PTX-regulated pathway(s) that is negatively modulated by PKC, possibly at the level of PLC-β. In contrast, PAF activates eosinophils independent of Gi by a mechanism that is abolished by selective PKC inhibitors. Collectively, these results suggest that agonists can recruit multiple and parallel signalling pathways in guinea pig eosinophils that can ultimately evoke the same functional response. This observation provides a persuasive explanation for the difference in sensitivity of PAF-, C5a-, and LTB4-induced eosinophil responses to cyclic AMP-elevating agents reported in in vitro studies.15 Finally, these results showed for the first time that a receptor-operated event on the eosinophil is essential for PAF, C5a, and LTB4-induced eosinophil recruitment in vivo.

### Table 3. Summary of the Effects of Various Pharmacologic Interventions on Eosinophil Aggregation, Intracellular Ca2+ Transients, and Recruitment In Vivo Induced by PAF, C5a, and LTB4

<table>
<thead>
<tr>
<th>Intervention</th>
<th>PAF</th>
<th>C5a</th>
<th>LTB4</th>
</tr>
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<tbody>
<tr>
<td><strong>PTX (1 μg/mL)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca2+ transient</td>
<td>Unaffected</td>
<td>Abolished</td>
<td>Attenuated by ~90%</td>
</tr>
<tr>
<td>Aggregation</td>
<td>Unaffected</td>
<td>Abolished</td>
<td>Attenuated by ~75%</td>
</tr>
<tr>
<td>In vivo recruitment</td>
<td>Attenuated by ~50%</td>
<td>Abolished</td>
<td></td>
</tr>
<tr>
<td><strong>Ro 31-8220 (3 × 10^-5 mol/L)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca2+ transient</td>
<td>Abolished</td>
<td>Prolonged, no change in amplitude</td>
<td>Prolonged, no change in amplitude</td>
</tr>
<tr>
<td>Aggregation</td>
<td>Abolished</td>
<td>Unaffected or enhanced</td>
<td>Stimulated at high concentrations</td>
</tr>
<tr>
<td><strong>Staurosporine (10^-7 mol/L)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca2+ transient</td>
<td>Unaffected</td>
<td>Prolonged, no change in amplitude</td>
<td>Prolonged, no change in amplitude</td>
</tr>
<tr>
<td>Aggregation</td>
<td>Enhanced</td>
<td>Enhanced</td>
<td>Enhanced</td>
</tr>
<tr>
<td><strong>H-89 (10^-6 mol/L)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aggregation</td>
<td>Enhanced</td>
<td>Enhanced</td>
<td>Enhanced</td>
</tr>
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</table>

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Pertussis Toxin Shows Distinct Early Signalling Events in Platelet-Activating Factor−, Leukotriene B4−, and C5a-Induced Eosinophil Homotypic Aggregation In Vitro and Recruitment In Vivo

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