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 chemoattractant-induced eosinophil recruitment in vivo.

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Eosinophil aggregation. Aggregation experiments were performed as previously described. Briefly, guinea pig eosinophils were resuspended (5 \times 10^6 cells/mL) in PBS, CaCl_2 and MgCl_2 (final concentrations, 1.0 mmol/L and 0.7 mmol/L, respectively) and incubated to allow for 5 minutes at 37°C on a shaking platform. After five minutes before use, the cells were warmed to 37°C and aliquots (300 \muL) 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 \times 10^6 cells/mL in PBS with 0.25% bovine serum albumin [BSA]) were loaded with fura-2-acetoxymethyl ester (2.5 \mumol/L, 30 minutes at 37°C). After two washes, eosinophils were resuspended at 10^6 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-\muL 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. [Ca^{2+}]_i levels were calculated using the ratio of the two fluorescence readings and a k_d for Ca^{2+} 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 \(^{111}\text{In}\) as previously described. The \(^{111}\text{In}\)-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 \times 10^6 cells/animal) together with \(^{125}\text{I}\)-human serum albumin (\(^{125}\text{I}\)-HSA; 5 \muCi) 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^{-8} mol/site, LTb4 at 10^{-12} to 10^{-10} mol/site, and C5a at 10^{-12} to 10^{-11} mol/site) were administered in 0.1-mL volumes into the shaved dorsal skin following a randomized injection plan. \(^{111}\text{In}\)-labeled eosinophil accumulation and \(^{125}\text{I}\)-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 pentobarbital, 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 automated 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 \(^{111}\text{In}\)-eosinophils per skin site and \(^{125}\text{I}\)-HSA extravasation was expressed as microliters of plasma.

PTX treatment. Purified eosinophils were diluted in PBS (5 \times 10^6 cells/mL in PBS with 0.25% BSA and 10 mmol/L HEPES) and incubated with PTX (1 \mug/mL) at 37°C for 2.5 hours. An aliquot was separated and labeled with Fluor-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 \times 10^6 cells/mL for the aggregation experiments and 10^6 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, \(^{111}\text{In}\)-labeled eosinophils were diluted in PBS (5 \times 10^6 cells/mL in PBS with 0.25% BSA and 10 mmol/L HEPES) and incubated with PTX (1 \mug/mL) at 37°C for 2.5 hours. The cells were then washed twice and resuspended in a final volume of 5 \times 10^6 \(^{111}\text{In}\)-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). Staurosporine 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 transmembrane spanning receptors for PAF, C5a, and LTb4, by using the bacterial toxin, PTX. Eosinophils were pretreated for 2.5 hours with PTX (1 \mug/mL) or its vehicle and agonist-induced aggregation and Ca^{2+} mobilization were subsequently assessed. PAF, C5a, and LTb4 elicited the aggregation of guinea pig eosinophils in a concentration-dependent manner with approximate EC_{50} values of 2 \times 10^{-7} mol/L, 2 \times 10^{-8} mol/L, and 3 \times 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 Ca^{2+}-mobilization were unaffected in PTX-treated eosinophils, the same responses evoked by LTb4 were markedly reduced (75% to 90%) and those affected by C5a were abolished (Table 1).

Effects of PTX on the recruitment of \(^{111}\text{In}\)-eosinophil into guinea pig skin. In agreement with previous reports, \(^{111}\text{In}\)-intradermal injection of LTb4 (10^{-12} to 10^{-10} mol/site), PAF (10^{-11} to 10^{-9} mol/site), and C5a (10^{-12} to 10^{-11} mol/site) into the skin of guinea pigs induced a dose-dependent recruitment of \(^{111}\text{In}\)-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,\(^{19}\) 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, 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 of 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 125I-HSA in response to PAF, C5a, and LTB, 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, 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 10⁻¹⁰ 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, 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 Ca²⁺ mobilization. Pretreatment of eosinophils with the PKC inhibitor, Ro 31-8220, at a concentration (3 x 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, was markedly inhibited by Ro 31-8220 but was significantly enhanced when higher concentrations of the agonist were used (Fig 4C).

In contrast to the effects seen with Ro-31-8220, pretreatment of eosinophils with the nonspecific protein kinase inhibitor, staurosporine (10⁻⁷ mol/L), which inhibited PMA-induced aggregation by 76.6% ± 2.9% (n = 5), significantly augmented the same response evoked by PAF, C5a, and LTB, (Fig 5A). Because staurosporine is known to inhibit protein kinases in addition to PKC,²⁰ it was hypothesised that the augmentation of the aggregation response might be due to an effect on PKA that generally exerts an inhibitory
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></th>
<th>Aggregation (% maximal response induced by PMA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>PAF (10⁻⁸ mol/L)</td>
<td>16.3 ± 4.2</td>
</tr>
<tr>
<td>C5a (10⁻⁷ mol/L)</td>
<td>24.8 ± 2.5</td>
</tr>
<tr>
<td>LTB₄ (10⁻⁸ mol/L)</td>
<td>20.5 ± 1.2</td>
</tr>
<tr>
<td>PTX</td>
<td></td>
</tr>
<tr>
<td>PAF (10⁻⁸ mol/L)</td>
<td>13.3 ± 5.2</td>
</tr>
<tr>
<td>C5a (10⁻⁷ mol/L)</td>
<td>0*</td>
</tr>
<tr>
<td>LTB₄ (10⁻⁸ mol/L)</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.

FIG 3. Effect of PTX on the recruitment of ¹¹¹In-eosinophils in response to intradermal injection of C5a, LTB₄, or PAF. Eosinophils were pretreated for 2.5 hours with PTX (1 µg/mL, see the Materials and Methods) or vehicle. The cells were then labeled with ¹¹¹In and injected intravenously (2.5 × 10⁶ cells/animal) into recipient guinea pigs. ¹¹¹In-eosinophil recruitment was measured 1 hour after the intradermal injection of PAF (10⁻¹² to 10⁻¹⁰ mol/site), C5a (10⁻¹⁰ and 10⁻¹⁵ mol/site), and LTB₄ (10⁻¹⁰ to 10⁻¹⁵ mol/site). The dashed line across the graph represents background values in response to injection of saline. Results are the mean ± SEM of five pairs of animals.

FIG 4. Effect of the PKC inhibitor, Ro 31-8220, on eosinophil aggregation induced by (A) PAF, (B) C5a, or (C) LTB₄. Eosinophils were pretreated for 3 minutes with Ro 31-8220 (3 × 10⁻⁵ mol/L, open symbols) or vehicle (solid symbols) before the addition of PAF (10⁻¹⁰ mol/L to 10⁻⁷ mol/L), C5a (10⁻⁹ mol/L or 10⁻⁷ mol/L), or LTB₄ (10⁻¹⁰ mol/L to 10⁻⁷ mol/L). Results are expressed as the percentage of maximal aggregation induced by PMA (10⁻⁷ mol/L) and each point is the mean ± SEM for three to five experiments. **P < .01 when compared with control values.
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 \times 10^{-5} \text{mol/L}), or staurosporine (10^{-7} \text{mol/L}) before the addition of LTB4 (10^{-8} \text{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).

In contrast, pretreatment of eosinophils with staurosporine (10^{-7} \text{mol/L}) did not affect the Ca^{2+}/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 Ca^{2+}/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 Ca^{2+} concentration in guinea pig eosinophils were assessed. Collectively, the results presented herein strongly suggest that the early signalling events that ultimately promote aggregation and Ca^{2+}-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 Ca^{2+}-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. The results with C5a are similar to those reported by Thelen et al., who showed that C5a-induced Ca^{2+} mobilization and degranulation of human eosinophils were abolished by PTX. Similarly, activation of the NADPH oxidase by LTB4 in guinea pig eosinophils and the elaboration of arachidonic acid were markedly inhibited by PTX. Collectively, these data clearly indicate that agonism of C5a and, in large part, LTB4 receptors on guinea pig eosinophils involves the activation of one or more PTX-sensitive, heterotrimeric G-proteins. Recent studies have established that guinea pig and...
human eosinophils express a number of G-proteins that include Ga31, Goα, Goq11, and Goγ.24,25 Although it cannot be stated with certainty the type of G-protein involved in LTB4- and C5a-induced aggregation and Ca2+-mobilization, Gaα is unlikely to represent the PTX substrate because it is believed to be coupled primarily, although not exclusively (see Padrell et al26), to the activation of voltage-sensitive Ca2+ channels.27 Because Goα is not present in guinea pig eosinophils,25 a case can be made for Ga31/Gaα. 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 Gaα was not initially excluded. It was reasoned that the PTX insensitivity might occur if Gaα 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-α1. 

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, Gi1α (or Giα3) 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 LTB₄ 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 and Rooney et al) 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, 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, 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.

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. 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.

REFERENCES

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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>
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</thead>
<tbody>
<tr>
<td>PTX (1 μg/mL)</td>
<td></td>
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<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>Abolished</td>
</tr>
<tr>
<td>In vivo recruitment</td>
<td>Attenuated by ~50%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ro 31-8220 (3 × 10⁻⁵ mol/L)</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>Prolonged, no change in amplitude</td>
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<tr>
<td>Staurosporine (10⁻⁷ mol/L)</td>
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<td></td>
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<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>
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<tr>
<td>H-89 (10⁻⁸ mol/L)</td>
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<tr>
<td>Aggregation</td>
<td>Enhanced</td>
<td>Enhanced</td>
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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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