

**Table 1. Effect of PTX (1 µg/mL, 2.5 Hours at 37°C) on Eosinophil Aggregation Induced by PAF, C5a, and LTB<sub>4</sub>**

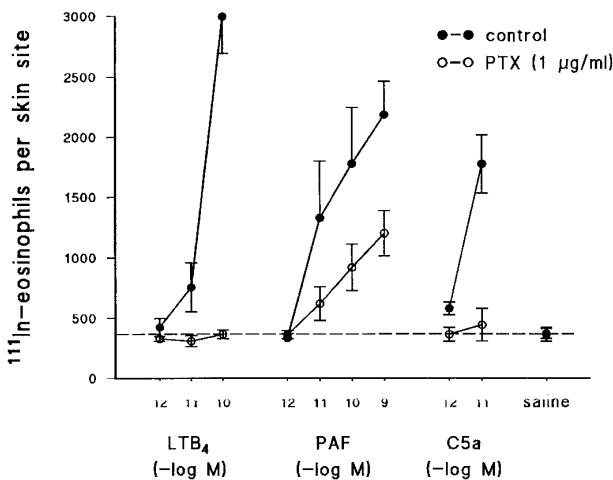
	Aggregation (% maximal response induced by PMA)	
	Control	PTX
PAF ( $10^{-8}$ mol/L)	16.3 ± 4.2	13.3 ± 5.2
C5a ( $10^{-7}$ mol/L)	24.8 ± 2.5	0*
LTB <sub>4</sub> ( $10^{-8}$ mol/L)	20.5 ± 1.2	5.0 ± 2.0*

Values are the mean ± SEM of three to four experiments.

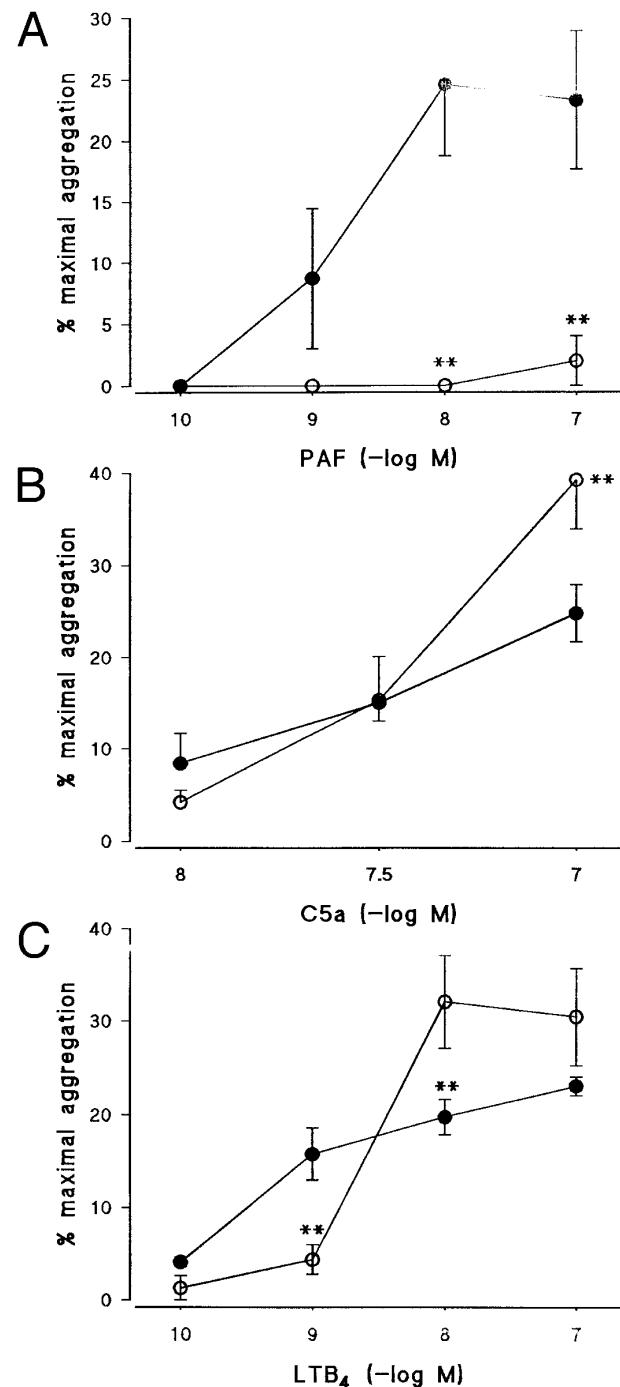
\* $P < .01$  when compared with control values.

effect in eosinophils (eg, Teixeira et al<sup>13</sup>). As shown in Fig 5B, the PKA inhibitor, H89, at a concentration ( $10^{-5}$  mol/L) shown previously to inhibit PKA in intact neutrophils,<sup>21</sup> significantly enhanced PAF-, C5a-, and LTB<sub>4</sub>-induced eosinophil aggregation.

*Effect of the protein kinase inhibitors Ro 31-8220 and staurosporine on Ca<sup>2+</sup> mobilization in eosinophils.* The effect of Ro 31-8220 and staurosporine on the Ca<sup>2+</sup> transient evoked by PAF, C5a, and LTB<sub>4</sub> 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 Ca<sup>2+</sup> signal. Whereas PAF-induced Ca<sup>2+</sup> mobilization was abolished in eosinophils pretreated with Ro 31-8220 ( $3 \times 10^{-5}$  mol/L), equivalent responses elicited by LTB<sub>4</sub> and C5a were significantly enhanced. This effect was due predominantly to an increase in the duration rather than the peak height of the Ca<sup>2+</sup> transient (see Fig 6 for a typical LTB<sub>4</sub> response). Lower concentrations of C5a and LTB<sub>4</sub> were affected similarly by pretreatment with Ro 31-8220 (data not shown). In



**Fig 3. Effect of PTX on the recruitment of <sup>111</sup>In-eosinophils in response to intradermal injection of C5a, LTB<sub>4</sub>, 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 <sup>111</sup>In and injected intravenously ( $2.5 \times 10^6$  cells/animal) into recipient guinea pigs. <sup>111</sup>In-eosinophil recruitment was measured 1 hour after the intradermal injection of PAF ( $10^{-12}$  to  $10^{-10}$  mol/site), C5a ( $10^{-12}$  and  $10^{-11}$  mol/site), and LTB<sub>4</sub> ( $10^{-12}$  to  $10^{-10}$  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) LTB<sub>4</sub>.** Eosinophils were pretreated for 3 minutes with Ro 31-8220 ( $3 \times 10^{-5}$  mol/L, open symbols) or vehicle (solid symbols) before the addition of PAF ( $10^{-10}$  mol/L to  $10^{-7}$  mol/L), C5a ( $10^{-8}$  mol/L or  $10^{-7}$  mol/L), or LTB<sub>4</sub> ( $10^{-10}$  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.