phospholipid, it was thought initially that inhibition was via sequestration of substrate. However, we subsequently demonstrated that p68, which binds both phosphatidylethanolamine and phosphatidylyserine, has a poor affinity for PC. It is likely, therefore, that phospholipase inhibition by p68 may involve a direct interaction between the two proteins.

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Inhibition of arachidonate lipoxygenase by ketotifen

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Ketotifen is commonly used for the prophylactic treatment of bronchial asthma. There is evidence that ketotifen antagonizes 5-hydroxytryptamine and acts by preventing mediator release from mast cells and protects asthmatic patients from allergen and or histamine-induced bronchoconstriction (Girard & Cuevas, 1977; Wuethrich et al., 1978). Data are also available supporting the view that long-term treatment of asthmatic patients with ketotifen reduces bronchial hyper-reactivity in such patients.

Platelet-activating factor is a potent inducer of platelet aggregation and mediates bronchoconstriction and non-specific bronchial hyper-reactivity. We recently found that ketotifen selectively inhibits platelet-activating-factor-induced human platelet aggregation (Saeed et al., 1988). On the other hand, it has been demonstrated that human lung tissue (asthmatic and normal) when incubated with arachidonic acid produces 12- and 15-hydroxyeicosatetraenoic acid via lipoxygenase pathways. Furthermore, these hydroxy acids were found to be strong bronchoconstrictor agents (Copas et al., 1982). In the present investigation, therefore, we have examined the effect of ketotifen on the production of lipoxygenase products by human platelets and rat lung.

Human blood platelets were routinely obtained in plastic bags containing 30–40 ml of concentrated platelet-rich plasma from the Aga Khan University Hospital Laboratory. The platelet-rich plasma was centrifuged at 1200 g for 20 min and the sedimented platelets were washed twice with an ice-cold solution of 0.15 M-NaCl. The washed platelet pellet was homogenized in 2 vol. of phosphate buffer (50 mM, pH 7.4). The homogenate was centrifuged at 1200 g for 20 min and 300 μl of the supernatant fraction (containing 0.4 mg of protein) was incubated with 0.1 μCi of [14C]arachidonic acid (specific activity 58.4 mCi/mmol; Amersham, U.K.) in the presence or absence of test drug in phosphate buffer in a final volume of 1.0 ml. After 15 min with gentle shaking in air at 37°C, the radioactive metabolites and unchanged substrate were extracted with 7 vol. of ethyl acetate. The organic phase was evaporated to dryness under a stream of nitrogen. Residues were dissolved in 40 μl of ethanol and 25 μl was applied to silica gel G t.l.c. plates (Analtech, Delaware, U.S.A.) that were developed in ether/petroleum ether (boiling range 40–60°C)/acetic acid (50:50:1, by vol.) to a distance of 17 cm. By use of this solvent system the various hydroxyeicosatetraenoic acids are separated with prostaglandins and thromboxane B2 remaining at the origin. Zones containing radioactivity were located and quantified by use of a Berthold t.l.c. linear analyser and chromatography data system (model LB 5 11, Berthold, W. Germany).

Experiments with rat lung were performed using the procedure outlined above except that homogenized lung tissue was used instead of platelets. Protein concentration was determined by the method of Lowry et al. (1951) with human serum albumin as a standard.

Incubation of [14C]arachidonic with the 1200 g supernatant fraction of the platelet homogenate resulted in the likely, therefore, that phospholipase inhibition by p68 may involve a direct interaction between the two proteins.

Fig. 1. Radiochromatograms of the products formed when [14C]arachidonic acid was incubated with the 1000 g supernatant fraction of human platelets

(a) Control.
(b) Control + 0.1 mM-5-hydroxytryptamine.
(c) 5-Hydroxytryptamine + 0.16 mM-indomethacin.
(d) 5-Hydroxytryptamine + 0.1 mM-nordihydroguaiaretic acid.

Solvent system: diethyl ether/petroleum ether (boiling range 40–60°C)/acetic acid (50:50:1, by vol.). Abbreviations: AA, arachidonic acid; PG, prostaglandin; THETE, trihydroxy-eicosatetraenoic acid; 12-HETE, 12-hydroxy-eicosatetraenoic acid.
formation of various lipoxygenase products. A typical radio-
chromatographic scan obtained after t.l.c. of the products of
incubation is shown in Fig. 1. The lipoxygenase products
were identified by co-migration with authentic hydroxy acid
standards. A characteristic pattern of one major ($R_t$ 0.6) and
one minor ($R_t$ 0.15) peak can be seen (Fig. 1). The major
compound co-migrated with 12-hydroxyeicosatetraenoic
acid. When 5-hydroxytryptamine (1–100 μM) was added to
the incubation mixture, mean 12-hydroxyeicosatrienoic
acid production exceeded the control value by 1.15-fold to
4.1-fold in a concentration-related manner ($P<0.001,
n = 7$). Addition of nordihydroguaiaretic acid, an inhibitor of
lipoxygenase activity, and ketotifen to the incubation mixture
caus ed a concentration-dependent inhibition of the conver-
sion of arachidonic acid to lipoxygenase products by human
platelets. The mean values ($\pm S.E.M.$) for inhibiting the pro-
duction of 12-hydroxyeicosatetraenoic acid by 50% ($IC_{50}$)
for ketotifen and nordihydroguaiaretic acid were 79 ± 9 and
35 ± 4 μM, respectively. Indomethacin (up to 200 μM), an
inhibitor of fatty acid cyclo-oxygenase activity, had no inhibi-
tory effect on 5-hydroxytryptamine-induced stimulation of
12-hydroxyeicosatetraenoic acid production, whereas it sim-
ulated the production of trihydroxyeicosatrienoic acid ($R_t$
0.15) 3-fold. In separate experiments we have found that ket-
otifen also inhibits rat lung arachidonate lipoxygenase and
soybean lipoxygenase activities.

These experiments demonstrate that ketotifen inhibits
arachidonate lipoxygenase activity in platelets. Since 12-
hydroxyeicosatetraenoic acid and its labile precursor 12-
hydroperoxoicosatetraenoic acid are potent chemotactic
and vasoactive agents (Goetzl & Sun, 1979), we suggest that
ketotifen may directly influence 5-hydroxytryptamine-
induced stimulation of platelet arachidonate lipoxygenase
activity.

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Differential effects of anticholinergic drugs on platelet aggregation induced by platelet-activating
factor and other agents

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It has been demonstrated that human blood platelets possess
an adrenergic receptor of the $\alpha_2$-subtype, which has been
well characterized by both functional (Grant & Scrutton,
1980) and radioligand-binding (Alexander et al., 1978;
Daiguji et al., 1981) studies. There is also evidence that
adrenergic blocking drugs inhibit platelet aggregation
induced by adrenaline and noradrenaline both in vitro and in
vivo (Suria et al., 1986). More recently, we have reported
evidence that human platelets also contain muscarinic
cholinergic receptors (Saeed et al., 1988). In the present
communication we describe the findings of additional inves-
tigation demonstrating that anticholinergic drugs selectively
inhibit platelet aggregation induced by platelet-activating
factor.

The effects of various anticholinergic and other drugs on
platelet aggregation were studied as follows. After vene-
 puncture of normal volunteers who had not taken any medi-
cation for at least 7 days, blood was collected into siliconized
glass tubes containing 1 vol. of sodium citrate [3.8%, w/v].
Platelet-rich plasma was separated by centrifugation at 260 g
for 15 min. Platelet-poor plasma was prepared by centri-
figation of blood at 3000 g for 10 min. Platelet count was deter-
mined by phase contrast microscopy and all aggregation
studies were conducted at 37°C with platelet-rich plasma
having platelet counts between 2.5–3.0 x 10^5/ml of plasma.
Aggregation was monitored with a Dual-Channel Lumi-

Table 1. Effect of anticholinergic drugs and indomethacin on platelet aggregation induced
by various aggregating agents

<table>
<thead>
<tr>
<th>Drug</th>
<th>Aggregating agents…</th>
<th>Platelet-activating factor</th>
<th>ADP</th>
<th>Arachidonic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atropine</td>
<td>102 ± 7</td>
<td>705 ± 5</td>
<td>n.i.</td>
<td>n.i.</td>
</tr>
<tr>
<td>Homatropine</td>
<td>114 ± 2.5</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
</tr>
<tr>
<td>Himbacine</td>
<td>94 ± 5</td>
<td>405 ± 5</td>
<td>n.i.</td>
<td>n.i.</td>
</tr>
<tr>
<td>Scopolamine</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
</tr>
<tr>
<td>Pirenzepine</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
</tr>
<tr>
<td>Hexamethonium</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
</tr>
<tr>
<td>d-Tubocurarine</td>
<td>26 ± 2</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
</tr>
<tr>
<td>Gallamine</td>
<td>10 ± 2</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>9 ± 0.7</td>
<td>35 ± 2</td>
<td>n.i.</td>
<td>n.i.</td>
</tr>
</tbody>
</table>

All values are means ± S.E.M. of three determinations. Assay conditions were as described
in the text. Abbreviation: n.i., not inhibited.