A comparative study of the effects of local anaesthetics on platelet aggregation

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A variety of drugs that are generally thought to modify biological membrane action may affect platelet function [1]. Phospholipase A₂ is a membrane-bound enzyme, and plays a key role in the release of arachidonic acid from platelet membrane phospholipids [2]. The arachidonic acid thus released is converted by platelet cyclo-oxygenase and lipoxygenase pathways to prostaglandin endoperoxides, thromboxane A₁ and hydroperoxyeicosatetraenoic acid, resulting in platelet aggregation. In the present investigation we sought to evaluate the effect of local anaesthetic agents (thought to act by stabilizing membrane) on platelet aggregation induced by various aggregating agents.

Blood was taken by venepuncture from normal human volunteers reported to be free of medication for 1 week. Blood samples were mixed with 3.8% (w/v) sodium citrate solution (9:1) and centrifuged at 260 g for 15 min at 20°C to obtain platelet-rich plasma. The remaining blood sample was centrifuged at 1200 g for 10 min to obtain platelet-poor plasma. Platelet count was determined by phase-contrast microscopy and all aggregation studies were carried out at 37°C with platelet-rich plasma having platelet counts between 2.5 and 3.0 × 10⁵/ml of plasma. Aggregation was monitored with a Dual-channel Lumi-aggregometer (model 400, Chronolog Corporation, Chicago, IL, U.S.A.) using 0.45 ml aliquots of platelet-rich plasma. The final volume was made up to 5 ml with sodium chloride (0.9%, w/v) or test drug and incubated for 1 min before challenge with the aggregating agent. Aggregation was induced with ADP (2.2 µM), sodium arachidonate (1.7 mM) or platelet-activating factor (0.8 µM). The resulting aggregation was recorded with a Lumi-aggregometer and expressed as percentage inhibition compared with control at 4 min after challenge. All drugs were tested at three to six different concentrations in duplicate. Statistical differences between control and drug-treated platelet preparations were determined by Student's t-test.

Table 1 summarizes the effects of various local anaesthetic drugs on platelet aggregation induced by ADP, arachidonic acid and platelet-activating factor. All local anaesthetic agents studied inhibited platelet-activating-factor-induced aggregation in a concentration-related manner. The order of potency, in increasing fashion, was benzocaine, lignocaine, benoxinate, tetracaine, alcaine and procaine with concentrations producing 50% inhibition of platelet aggregation (IC₅₀) of 240, 235, 170, 100, 80 and 78 µM, respectively. Only alcaine, benoxinate and procaine were found to have weak inhibitory effects against arachidonic acid-induced platelet aggregation, with IC₅₀ values of 250, 200, and 180 µM, respectively. Similarly ADP-induced aggregation was inhibited by procaine and tetracaine only.

Our data demonstrated that all local anaesthetic drugs studied exert a strong inhibitory effect against platelet-activating-factor-induced aggregation. Previously, general anaesthetics have been reported to have an inhibitory effect on platelet aggregation induced by ADP [3]. Furthermore, we have recently described a selective, inhibitory effect of anti-arrhythmic drugs against platelet-activating-factor-induced aggregation [4]. Taken together, these findings suggest that the inhibitory effect of local anaesthetic and anti-arrhythmic drugs on platelet aggregation may be related to their ability to stabilize or penetrate the lipid monolayer in the plasma membrane.

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Table 1. Inhibition of human platelet aggregation by local anaesthetics

All values are means ± S.E.M. of three to six experiments. Assay conditions were as described in the text. Statistical significance was assessed by Student's t test. Abbreviation: n.i., not inhibited up to 500 µM.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Aggregating agent</th>
<th>ADP IC₅₀ (µM)</th>
<th>Arachidonic acid IC₅₀ (µM)</th>
<th>Platelet-activating factor IC₅₀ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcaine</td>
<td>n.i.</td>
<td>250 ± 8</td>
<td>80 ± 5</td>
<td></td>
</tr>
<tr>
<td>Benoxinate</td>
<td>n.i.</td>
<td>200 ± 9</td>
<td>170 ± 8</td>
<td></td>
</tr>
<tr>
<td>Benzocaine</td>
<td>n.i.</td>
<td>240 ± 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lignocaine</td>
<td>n.i.</td>
<td>235 ± 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Procaine</td>
<td>300 ± 10</td>
<td>180 ± 6</td>
<td>78 ± 5</td>
<td></td>
</tr>
<tr>
<td>Tetracaine</td>
<td>182 ± 7</td>
<td>n.i.</td>
<td></td>
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</table>
We thank Mr Hazratuddin Khan for technical assistance.


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**Comparison of the rate of occupancy of receptors by anti-cholinergic drugs**

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The physiological role of acetylcholine is mediated through activation of either muscarinic or nicotinic receptors. The muscarinic responses to acetylcholine that are blocked by atropine are further subdivided into two types based on the antagonist affinities for muscarinic receptors in different tissues [1]. Receptors which are blocked by low concentrations of pirenzepine, an anti-muscarninic drug, are called as neuronal (M₃) and those which are insensitive to low concentrations of pirenzepine peripheral (M₂) receptors. Peripheral muscarinic receptors are further subdivided into cardiac and smooth muscle receptors as himbacine, a plant-derived alkaloid, exhibits at least 10-fold higher affinity for cardiac muscarinic receptors [2]. We have recently proposed that himbacine may be a pharmacological tool for studies involving heterogeneity of muscarinic receptors in cardiac and smooth muscle [3]. However, the rate of occupancy of muscarinic receptors for himbacine is yet to be determined.

In this investigation, the onset of action of himbacine was studied and compared with that of atropine and/or homatropine.

The onset of antagonism of himbacine and atropine was studied in isolated guinea-pig ileum and atria. In ideal experiments, when responses were stable to acetylcholine the bathing fluid was replaced with Tyrode's solution containing an antagonist. (a) The time course of development of antagonism of acetylcholine by himbacine and atropine was studied in five ideal preparations and the results are shown in Fig. 1(a). Both himbacine (1 μM) and atropine (10 μM) at equi-effective concentrations commenced antagonizing the contractile response to acetylcholine immediately after contact with the tissue, but the time required to achieve maximum antagonism varied between the drugs. In three out of five experiments, himbacine exerted its maximum antagonistic effect almost immediately, whereas in the other two preparations it took 5-10 min for complete antagonism. (b) Comparison of the onset of action of himbacine and atropine in guinea-pig ileum. The symbols represent five different preparations. Ordinate: response to acetylcholine relative to that reached at equilibrium; abscissa: time after replacing bathing fluid with one containing antagonist. (b) Representative traces showing reversal of carbachol responses by himbacine, homatropine and atropine in guinea-pig left atria driven electrically. At the concentrations shown for the different antagonists, the magnitude of the effects, in terms of agonist dose-ratios, were comparable. The symbols represent times when drugs were added to the tissue bath.

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