A possible way in which extracellular Ca\(^{2+}\) may modulate the intracellular flux of Ca\(^{2+}\) is via an effect on platelet cyclic nucleotide metabolism.


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**Studies on the role of cyclic GMP in the control of human platelet function**

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Although it is widely accepted that an increase in cyclic AMP production results in an inhibition of platelet function (Mills & Smith, 1971; Best et al., 1977) little is known concerning the role of cyclic GMP in the platelet. In several tissues, an increase in cyclic GMP often occurs under conditions that oppose an increase in cyclic AMP (Goldberg et al., 1973) and it has been suggested that platelet aggregation may be accompanied by an increase in cyclic GMP concentrations in response to several agents (Jakobs et al., 1974; Davies et al., 1976; Glass et al., 1977). In addition, Chiang et al. (1976) have provided evidence that platelet aggregation was enhanced in the presence of cyclic GMP.

To further examine the possibility that cyclic GMP may be a messenger in the initiation of platelet function, we have studied the conditions under which changes in cyclic GMP occur and also investigated the effects of cyclic GMP on certain aspects of platelet function. Cyclic GMP was measured by a specific and sensitive radioimmunoassay in suspensions of washed human platelets in Tris/saline buffer. The aggregating agent arachidonate produced maximally a 3-fold increase in cyclic GMP over basal values in the presence of the phosphodiesterase inhibitor, isobutylmethylxanthine (Table 1). If the metabolism of arachidonate to prostaglandin endoperoxides was a prerequisite for cyclic GMP production. The non-aggregatory fatty acid dihomo-\(\gamma\)-linoleate produced a similar increase in platelet cyclic GMP (Table 1), suggesting that an increase in cyclic GMP production may be associated with the peroxidation of unsaturated fatty acids. In support of this hypothesis, stearate was found to have no significant effect on platelet cyclic GMP concentrations. Prostaglandins \(E_1, E_2\) and \(F_2\) were also without effect.

The effects of cyclic GMP and dibutyl cyclic GMP were examined on platelet aggregation and on 5-hydroxy\(\delta\)-tryptamine secretion. Preincubation of platelets for 2min in the presence of dibutyl cyclic GMP (0.1–1.0mM) inhibited platelet aggregation and 5-hydroxytryptamine secretion in response to ADP, adrenaline or collagen. Cyclic GMP produced a similar, though less potent, inhibition of platelet function.

In general, the inhibitory effect of dibutyl cyclic GMP was comparable with, though less marked than, that of dibutyl cyclic AMP. This suggests that cyclic GMP may inhibit platelet aggregation and secretion by mimicking cyclic AMP and thus by activating a cyclic AMP-dependent protein kinase (Pareti et al., 1978). Cyclic AMP is known to inhibit the production by platelets of thromboxane \(B_2\), probably by limiting the availability of endogenous arachidonate (Minkes et al., 1977). We found that both cyclic AMP and cyclic GMP were able to inhibit thromboxane \(B_2\) production by washed human platelets in response to thrombin. Thus it is possible that cyclic GMP, in common with cyclic AMP, may impair platelet function at least in part by inhibiting the liberation of arachidonate from platelet phospholipid.

In conclusion, our results indicate that platelet cyclic GMP concentrations were not related to aggregation or secretion and thus cyclic GMP is probably not an important messenger in platelet activation. Furthermore, cyclic GMP was found to be a weak inhibitor of platelet aggregation, secretion and thromb-
oxane biosynthesis. This may be a relatively non-specific effect shared with a wide spectrum of cyclic nucleotides and their derivatives.


Effects of \( D \)- and \( L \)-octopamine and of pharmacological agents on the metabolism of locust flight muscle

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The monoamine octopamine has been shown to be widely distributed in the nervous tissues of invertebrates (see Robertson & Juorio, 1976), and some of its effects resemble those of catecholamines in vertebrates. Previous experiments (Candy, 1978) have shown that the oxidation of substrates by perfused locust flight muscle is stimulated by \( D \)-octopamine. However, it was not established whether the octopamine receptors were specific for the \( D \) or the \( L \)-form of the amine. Since locust haemolymph has now been shown to contain the \( D \)-isomer (Goosey & Candy, 1980), it was important to establish whether the muscles respond to \( D \)-octopamine. Results of experiments designed to test this are reported in the present communication.

Octopamine also has a modulatory action on the myogenic rhythm of locust leg muscle (Evans & O'Shea, 1978) and can stimulate adenylate cyclase from lobster blood cells (Batelle & Kravitz, 1978) and cockroach brain (Harmer & Horn, 1977). The receptors of these tissues resemble the \( \alpha \)-adrenergic receptors of mammals, as shown by effects of drugs that are known to selectively block \( \alpha \) or \( \beta \) type responses in mammalian systems. We now report the effects of such drugs on the oxidative metabolism of locust flight muscle.

The oxidation of \( [U^{14}C] \)glucose to \( ^{14}C \)O\(_2\) by working locust (\( Schistocerca americana \) gregaria) thoracic muscle was followed by methods previously described (Candy, 1970, 1978). A mixed substrate of 0.08 \( \mu \)mol \( [U^{14}C] \)glucose and 0.1 \( \mu \)mol butyrate (unlabelled) was used, and perfusion was performed until the rate of appearance of \( ^{14}C \)O\(_2\) reached a steady state (60–80min). This was taken as the control rate of oxidation. The compound under test was then added to the perfusion medium, and the oxidation rate was followed for a further 30min. Where appropriate, \( D \)-octopamine was then added and the perfusion was continued for 30min.

The method of Kappe & Armstrong (1964) was used to obtain \( D \)-octopamine and \( L \)-octopamine.

The effects of different concentrations of \( D \)-octopamine on the perfused muscles were tested. A half-maximum response was obtained with approx. 0.7 \( \mu \)mol \( D \)-octopamine. At 0.15 \( \mu \)mol (corresponding to the concentration of \( D \)-octopamine in haemolymph after a few minutes' flight; Goosey & Candy, 1980) the control oxidation rate of 0.37 \( \pm \)0.02 \( \mu \)mol of glucose oxidized/min per mg of muscle increased to 0.51 \( \pm \)0.03 after addition of \( D \)-octopamine (mean \( \pm \)S.E.M., \( n = 9 \), \( P < 0.001 \)). This establishes that physiological concentrations of \( D \)-octopamine are capable of stimulating oxidative metabolism in flight muscle.

The \( L \)-isomer of octopamine also stimulated oxidation of glucose by the muscles, but approximately 10-fold as much \( L \)-octopamine was required to give the same stimulation as a given concentration of \( D \)-octopamine. The octopamine receptor therefore appears to show stereospecificity for \( D \)-octopamine, in keeping with the presence of the \( D \)-isomer in locust haemolymph. Harmer & Horn (1977) have shown that the adenylyl cyclase from cockroach brain is stimulated by \( D \)-octopamine to a much greater extent than \( L \)-octopamine.

The \( \beta \)-blockers propranolol and dichloroisoproterenol do not markedly inhibit octopamine stimulation of oxidation (Table 1), but the \( \alpha \)-blocker phenotamine almost completely inhibits the action of octopamine. However, phenoxybenzamine stimulated muscle metabolism in the absence of octopamine and failed to

Table 1. Effects of \( D \)-octopamine and drugs on the oxidation of \( [U^{14}C] \)glucose by perfused locust thoracic muscle

<table>
<thead>
<tr>
<th>Drug</th>
<th>Type</th>
<th>+ Test compound (( D )-Octopamine (1 ( \mu )mol))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propranolol (100 ( \mu )mol)</td>
<td>( \beta )-Antagonist</td>
<td>9 ( \pm ) 8</td>
</tr>
<tr>
<td>Dichloroisoproterenol (100 ( \mu )mol)</td>
<td>( \beta )-Antagonist</td>
<td>18 ( \pm ) 9</td>
</tr>
<tr>
<td>Phenoxybenzamine (100 ( \mu )mol)</td>
<td>( \alpha )-Antagonist</td>
<td>35 ( \pm ) 12</td>
</tr>
<tr>
<td>Phentolamine (10 ( \mu )mol)</td>
<td>( \alpha )-Antagonist</td>
<td>2 ( \pm ) 2</td>
</tr>
<tr>
<td>Isoproterenol (10 ( \mu )mol)</td>
<td>( \beta )-Agonist</td>
<td>12 ( \pm ) 12</td>
</tr>
<tr>
<td>Methoxamine (10 ( \mu )mol)</td>
<td>( \alpha )-Agonist</td>
<td>67 ( \pm ) 25</td>
</tr>
<tr>
<td>D-Octopamine (50 ( \mu )mol)</td>
<td></td>
<td>100 ( \pm ) 9 (5)</td>
</tr>
<tr>
<td>D-Octopamine (1 ( \mu )mol)</td>
<td></td>
<td>65 ( \pm ) 11</td>
</tr>
<tr>
<td>D-Octopamine (0.15 ( \mu )mol)</td>
<td></td>
<td>39 ( \pm ) 6 (9)</td>
</tr>
<tr>
<td>D-Octopamine (0.03 ( \mu )mol)</td>
<td></td>
<td>14 ( \pm ) 5 (7)</td>
</tr>
</tbody>
</table>