hormone-sensitive lipase, although a role for \( \text{Ca}^{2+} \) in some capacity cannot be excluded. The decreased lipolysis cannot be accounted for by a diminished response to cyclic AMP, but the lack of ready reversal of the effects on lipolysis, and the lowering of cell ATP content, suggest that a large part of the effects of both EGTA and ionophore A23187 on lipolysis may result from damage to fragile cells.

We thank Mrs. Margaret Wisbey for her excellent assistance, the British Diabetic Association for financial support, and Eli Lilly for the gift of ionophore A23187.


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**Relationships Between the Effect of Adrenaline and Ionophore A23187 on Adenosine 3':5'-Cyclic Monophosphate and on Free Intracellular Calcium Ion Concentrations in Pigeon Erythrocyte 'Ghosts'**

ANTHONY K. CAMPBELL and ROBERT L. DORMER

*Department of Medical Biochemistry, Welsh National School of Medicine, Heath Park, Cardiff CF4 4XN, Wales, U.K.*

Nucleated erythrocytes such as those from birds and amphibians have received much attention recently as a model system for studying the interaction between the \( \beta \)-adrenergic receptor and adenylate cyclase (Lefkowitz et al., 1976). In pigeon erythrocytes intracellular \( \text{Ca}^{2+} \) concentrations within the expected physiological range appear to inhibit adenylate cyclase (Campbell & Siddle, 1976). Indirect evidence has been obtained by a number of workers that changes in intracellular \( \text{Ca}^{2+} \) may play an important role in the action and secretion of certain hormones (Rasmussen et al., 1972; Berridge, 1976). At present, there is no technique available for measuring directly changes in free intracellular \( \text{Ca}^{2+} \) in small cells, although this has been achieved in some giant cells after the injection of \( \text{Ca}^{2+} \)-activated photoprotein aequorin (Blinks et al., 1976). Obelin, a photoprotein available in the United Kingdom and similar to aequorin, has been incorporated into pigeon erythrocyte 'ghosts' (Campbell & Dormer, 1975). These 'ghosts', which retain their nuclei, can be prepared relatively impermeable to \( \text{Ca}^{2+} \). The aim of the present report is to describe the preparation and characterization of hormone-sensitive erythrocyte 'ghosts' with particular reference to (a) their ATP, ionic and obelin contents, (b) their permeability to macromolecules and \( \text{Ca}^{2+} \), and (c) their sensitivity to \( \beta \)-adrenergic agonists and antagonists.

Pigeon erythrocytes were haemolysed in media at two osmolarities, (i) 6mM-NaCl/3 mM-MgCl₂, pH 7.0, or (ii) 6mM-NaCl/3 mM-MgCl₂/50mM-Tes, pH 7.0, and sealed erythrocyte 'ghosts' containing ATP and obelin prepared (Campbell & Dormer, 1975). The sealed 'ghosts' initially haemolysed in the higher-osmolarity medium more closely resembled intact cells in their ATP and ionic composition (K⁺, Na⁺ and Mg²⁺) than did 'ghosts' originally haemolysed in the lower-osmolarity medium. The percentage recovery of obelin trapped in the 'ghosts' was higher than that in the medium, suggesting that the free \( \text{Ca}^{2+} \) concentration inside the 'ghosts' was lowered during resealing, consistent with the presence of an active ATP-requiring \( \text{Ca}^{2+} \)-extrusion mechanism.

* Abbreviation: Tes, 2-(2-hydroxy-1,1-bis(hydroxymethyl)ethyl)amino)ethanesulfonic acid.
Pigeon erythrocytes were haemolysed in 6 mM-NaCl/3 mM-MgCl₂/50 mM-Tes, pH 7.0, and resealed for 60 min at 37°C in 150 mM-KCl/6 mM-NaCl/2 mM-MgCl₂/10 mM-Tes/2 mM-ATP (disodium salt)/10 mM-phosphoenolpyruvate (potassium salt) as previously described (Campbell & Dormer, 1975). Portions of the final 'ghost' suspension were incubated for up to 15 min at 37°C in 140 mM-NaCl/5 mM-KCl/2 mM-MgCl₂/1 mM-CaCl₂/10 mM-Tes, pH 7.4, in the absence of hormones (●) and in the presence of 5.5 μM-L-adrenaline (●) and 5.5 μM-adrenaline + 100 μM-5-hydroxytryptamine (▲). Cyclic AMP was extracted and measured by radioimmunoassay (Campbell & Siddle, 1976). Results are means ± S.E.M. (bars) for three determinations.

L-Adrenaline, L-noradrenaline and L-isoproterenol (0.55–165 μM) stimulated the formation of cyclic AMP inside the 'ghosts', the sensitivity of the 'ghosts' to these β-agonists being similar to that in intact cells. Cyclic AMP formation was only weakly stimulated by phenylephrine (55 μM). The 'ghosts' prepared in high-osmolarity medium produced up to ten times as much cyclic AMP as did those prepared in the low-osmolarity medium; however, the sensitivity to different concentrations of adrenaline appeared to be similar. The cyclic AMP concentration in 'ghosts' stimulated by adrenaline increased up to 15 min, little further increase being observed between 15 and 30 min (Fig. 1). The effect of adrenaline could be inhibited by propanolol, 5-hydroxytryptamine (Fig. 1) and the bivalent-cation ionophore A23187. Ionophore A23187, under the same conditions, caused a rapid increase in free intracellular Ca²⁺, as shown by an increase in obelin luminescence.

'Ghosts' relatively impermeable to Ca²⁺ could be prepared after 30–60 min sealing at 37°C. Initial haemolysis at high osmolarity resulted in 'ghosts' less permeable to Ca²⁺ than 'ghosts' originally haemolysed at low osmolarity (Fig. 2). L-Adrenaline (0.55–55 μM) decreased the rate of obelin luminescence in 'ghosts' which had been resealed for 30 min, but had little effect on 'ghosts' resealed for 60 min. The significance of this observation is uncertain at present, since β-adrenaline at the same concentrations as

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Pigeon erythrocytes were haemolysed in (i) 6mm-NaCl/3mm-MgCl₂ (○) or (ii) 6mm-NaCl/3mm-MgCl₂/50mm-Tes, pH7.0 (●). Sealed erythrocyte ‘ghosts’ were prepared in the presence of obelin as described in Fig. 1. The ‘ghosts’ were incubated at 37°C for 20s in 0.5ml of 140mm-NaCl/5mm-KCl/2mm-MgCl₂/10mm-Tes, pH7.4, and an equal volume of the same medium containing 2mm-CaCl₂ was then added. The rate of obelin luminescence was measured as counts recorded on a scalar and expressed as a percentage of the total activity trapped in the ‘ghosts’ (Campbell & Dormer, 1975). After 60s, 0.5ml of the same medium containing 1mm-CaCl₂ and 3% (v/v) Triton X-100 was added. Results are means of two determinations.

L-adrenaline also caused a decrease in obelin luminescence, whereas D-adrenaline had little or no effect on cyclic AMP formation. Further, the effect of adrenaline could not be inhibited by propranolol (10μM) or phentolamine (10μM). No effect of adrenaline on free obelin has yet been demonstrated. However, this possibility will require careful investigation before these results can be interpreted as showing an effect of adrenaline on free intracellular Ca²⁺.

These studies have shown that hormone-sensitive pigeon erythrocyte ‘ghosts’ can be prepared which are relatively impermeable to Ca²⁺. These ‘ghosts’ may provide a useful system for investigating the effects of some hormones and pharmacological agents on free intracellular Ca²⁺ and cyclic AMP in a sealed-membrane preparation.

We are greatly indebted to the Director and staff of the Marine Biological Association Laboratory, Plymouth. We thank Dr. K. Siddle for the gift of antibodies to cyclic AMP and Mr. M. E. T. Ryall for the construction of the luminescence apparatus. This work was supported by a grant from the Science Research Council and a grant from the British Diabetic Association to Professor C. N. Hales, whom we also thank for much encouragement and advice. Finally we thank Sterling-Winthrop Research Institute, New York, NY, U.S.A. for the kind gift of D-adrenaline.


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Ionophore- and Antigen-Induced Histamine Release from Mast Cells and a Comparison of their Inhibition

KEITH J. BARRETT-BEE

*Imperial Chemical Industries Ltd., Pharmaceuticals Division, Department of Biochemistry, Mereside, Alderley Park, Macclesfield, Cheshire, U.K.*

Ionophores are lipophilic compounds that have been shown to transport ions across biological and model membranes down a chemical gradient, i.e. they induce movement of an ion from a region of higher to one of lower concentration. This movement will depend on the concentration difference and presumably the relative binding constants of ionophores and ion for each other in the lipid and aqueous phases.

The bivalent metal ionophores A23187 and X537A have been used extensively over the past few years in the study of systems that require Ca^2+ for secretory activity, e.g. neurohypophyseal hormones (Nordmann & Currell, 1975) and noradrenaline from synaptosomes (Colburn et al., 1975). Garland & Mongar (1976) have shown that non-cytolytic release of histamine can be induced by the addition of ionophore A23187 or X537A to a suspension of mast cells. This release of histamine was inhibited by NaCN or by allowing the reaction to take place at 4°C, but not by disodium cromoglycate or by the addition of dibutyryl cyclic AMP at concentrations that inhibit the antigen-stimulated release of histamine (Garland & Mongar, 1976).

The observations reported below demonstrate the feasibility of inducing release of histamine from mast cells by addition of other ionophores that are specific for univalent ions. The release of histamine can be blocked by some inhibitors of antigen-stimulated release.

**Experimental**

Mast cells were prepared as previously reported (Barrett-Bee & Henderson, 1976) and incubated at 37°C in a modified Tyrode's buffer. Additions of ionophores were made in small volumes of ethanol (1%), and the cells were incubated for 10 min; they were then removed by centrifugation. The supernatant (1 ml) was treated with HClO₄ (0.1 ml of 4 M) and centrifuged to remove any precipitated protein; the histamine in the supernatant was determined by an automated fluorimetric technique. Total histamine in identical samples was determined by boiling the cells in HClO₄ (0.4 M) for 10 min; the degree of histamine release in test samples was expressed as a percentage of this value.

Table 1 shows the percentage release of histamine by several ionophoric uncouplers of oxidative phosphorylation. Clearly, histamine release can be induced under conditions where bivalent metal ions are not expected to be transported into cells. Under these conditions the cells were viable, as judged by Trypan Blue and Erythrocin B stains. It appears from these results that an internal acidification together with an efflux of K⁺ ions may be important in the release mechanism.

These findings prompted us to undertake further studies in which the ion-dependence of histamine release caused by ionophores A23187 and X537A is examined, the results of these studies are shown in Table 1(b). Both ionophores induced histamine release when Ca^2+ ions were present in the medium, but release induced by ionophore X537A showed a dependence on external K⁺ (5 mM); Ca^2+ ions inhibited this effect. Ionophore A23187 was able to release histamine in the absence of Ca^2+ ions when K⁺ ions were present. This may mean that ionophore A23187 is not specific for bivalent metal ions and may act as a potassium ionophore in this system, in a similar way to the