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Adrenergic receptors on monocytes modulate complement component synthesis

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SUMMARY

The addition of adrenaline, noradrenaline or phenylephrine, but not isoprenaline to monocyte cultures enhanced synthesis of the second complement component (C2). This effect was abrogated by the concomitant addition of the receptor antagonist, phentolamine, but not the β receptor antagonist propranolol. Thus the receptor involved is an α adrenergic receptor. Further studies showed that the receptor was of the α_1 subclass as prazosin inhibited the action of adrenergic agonists. Pulse label studies using ^{3}H-amino acids showed that the enhancement of synthesis of eight complement components (C2, C3, C4, C5, factor B, properdin, β1H and C3b inactivator) and total protein synthesis were also increased. The possible mechanisms underlying these changes are discussed.

INTRODUCTION

Catecholamines modulate a diverse array of cellular functions, by means of their interactions with receptors on cell membranes. The use of synthetic agonists and antagonists has shown that there are two classes of adrenergic receptors, alpha (α) and beta (β), and that both classes can be further subdivided into α_1 and α_2, and β_1 and β_2 receptors (Hoffman & Lefkowitz, 1980). The interaction of adrenergic agonists with α receptors leads to inhibition of adenylcyclase activity with reduction in intracellular cAMP levels. Conversely the interaction of the adrenergic ligand with the β receptor stimulates adenylcyclase activity with a consequent increase in intracellular cAMP concentrations (Rodbell, 1980). We have previously shown that when the levels of cAMP in human monocytes, in culture, are increased, their capacity to produce the second complement component (C2) is reduced (Lappin & Whaley, 1981). The events occurring at the monocyte plasma membrane, which modulate adenylcyclase activity and C2 production require definition in order to understand the mechanisms by which the synthesis of complement components by these cells is controlled. In this paper we show that the synthesis of complement components by monocytes is enhanced by catecholamines acting on α_1 adrenergic receptors.

MATERIALS AND METHODS

Reagents. Adrenaline, noradrenaline, phenylephrine, isoprenaline, L-leucine, L-lysine, L-tyrosine, L-phenylalanine, L-proline (Sigma), Ficoll 400, (Pharmacia), sodium hypaque (Winthrop Laboratories), ^{3}H-amino acid mixture, (TRK 500 containing leucine, lysine, tyrosine, phenylalanine and proline; Radiochemical Centre, Amersham), Unisolve 1 (Koch Light) sodium

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deoxycholate, salicylsulphonic acid (SSA) sodium dodecyl sulphate (SDS), sodium hydroxide, (BDH), RPMI 1640, Linbro multiwell tissue culture plates, (Flow Laboratories), fetal calf serum, antibiotic-antimycotic solution, L-glutamine and 7·5% sodium bicarbonate (GIBCO Biocult) were obtained from the sources shown. Yohimbine, prazosin and clonidine were gifts from Dr F. Boyle (Department of Pharmacology, University of Glasgow).

Production of complement components and antisera. Human C1 (Lachmann & Hobart, 1978), C4 and C2 (Ruddy & Austen, 1967), C3 (Tack & Prahl, 1976), factor B (Hunsicker, Ruddy & Austen, 1973), properdin (Fearon & Austen, 1975) and β1H (Whaley & Ruddy, 1976) were purified as described.

Rabbit antisera to human C3, C4, factor B, properdin and β1H were prepared as described (Whaley, 1980). Rabbit anti-C2 was a gift from Professor P. J. Lachmann and goat anti-C3 was a gift from Dr D. Shultze. Goat anti-C3bINA was purchased from Flow Laboratories.

C2 haemolytic assay. C2 concentrations in monocyte culture supernatants were assayed using a stoichiometric haemolytic assay (Rapp & Borsos, 1970).

Monocyte cultures. Monocyte cultures were prepared from Ficoll-Hypaque separated mononuclear leucocytes isolated from the heparinized venous blood of normal volunteers (Lappin & Whaley, 1980). Monocyte monolayers were cultured at 37°C in a humidified 5% CO_2/air atmosphere in RPMI 1640 containing 20% heat inactivated (2 hrs at 56°C) fetal calf serum, 9mM $NaHCO_3$, 4mM L-glutamine, 100 μg/ml streptomycin, 100 units/ml penicillin and 250 ng/ml fungizone (RPMI-FCS). At the end of the culture period the supernatants were removed, the monolayer washed in warm RPMI 1640 and then lysed in 1 ml of 2% SDS. The DNA content of the lysates was then determined by spectrofluorimetry (Einstein, Schneeberger & Colten, 1976). All the results were corrected for the DNA content of the monolayers.

The effects of the addition of adrenergic agonists and antagonists on C2 production were studied by adding the reagents to the cultures on day 0 and measuring C2 concentrations in the culture supernatants on day 7.

Incorporation of ^{3}H-amino acids into antibody precipitable radioactivity and acid precipitable radioactivity. The incorporation of radiolabel into newly synthesized protein was measured by culturing monocytes in the presence of 10 μCi ^{3}H-amino acid mixture for 7 days. On the 7th day of culture the amount of radioactivity precipitated by 10% SSA, and antisera to C2, C4, C3, C5, factor B, properdin, C3bINA and β1H was measured (Lappin, Moseley & Whaley, 1980).

Non-specific precipitation of ^{3}H-amino acids by antisera was reduced by washing precipitates three times (pH 7·2) in PBS containing 0·1% triton X100, 0·5% sodium decoxycholate, and L-leucine, L-lysine, L-tyrosine, L-phenylalanine and L-proline (all at a concentration of 10 mM). Precipitates were dissolved in 100 μl 1M NaOH, the solutions transferred to scintillation vials, emulsified in Unisolve 1, and counted using a Packard Tricarb scintillation counter, when chemiluminscence had subsided (Lappin *et al.*, 1980).

RESULTS

Effect of adrenergic agonists on C2 synthesis

Noradrenaline and adrenaline, which both act at α and β adrenergic receptors, enhanced C2 production, in a dose-dependent fashion (Fig. 1). Likewise, the selective α agonist, phenylephrine, enhanced C2 synthesis whereas isoprenaline, a selective β agonist had no effect on C2 synthesis (Fig. 1).

Effect of adrenergic receptor antagonists on enhancement of C2 synthesis produced by adrenergic agonists

Phentolamine (10^{-4} mol.$^{-1}$) abrogated the enhancement of C2 synthesis in cultures containing noradrenaline, adrenaline and phenylephrine (Fig. 2). The β receptor antagonist propranolol did not inhibit the enhancement of C2 synthesis induced by the agonists.

Effect of α_1 and α_2 receptor antagonists on phenylephrine-mediated enhancement of C2 synthesis

Prazosin (10^{-5} mol.l^{-1}) a selective α_1 receptor antagonist, totally abrogated the enhancement of C2

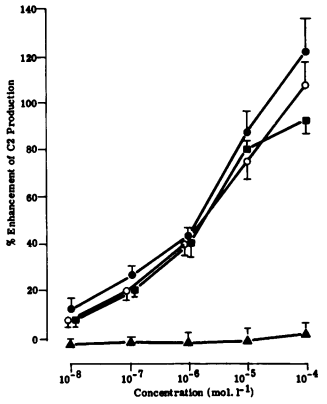

Fig. 1. Enhancement of monocyte C2 synthesis by adrenergic agonists (noradrenaline O–O; adrenaline ■–■; phenylephrine ●–●). Isoprenaline (▲–▲) did not affect C2 synthesis. C2 levels, expressed as percentage enhancement of untreated control cultures were measured on day 7. Each point represents the mean (±s.e.m.) of three experiments performed in duplicate on different donors.

synthesis produced by phenylephrine. In contrast yohimbine (10^{-5} mol.l^{-1}), a selective α_2 receptor antagonist was ineffective (Fig. 3a).

Clonidine, a selective α_2 receptor agonists potentiated C2 synthesis in a dose-dependent fashion (Fig. 3b). This action was prevented by prazosin (10^{-5} mol.l^{-1}) but not by yohimbine (10^{-5} mol.l^{-1}).

Incorporation of ^{3}H-labelled amino acids in acid and antibody precipitable radioactivity

Addition of phenylephrine to monocyte culture medium increased total protein synthesis as shown by increased incorporation of ^{3}H-amino acids into acid precipitable counts. Synthesis of C2, C4, C3, C5, factor B, properdin, C3bINA and β1H, was also increased as shown by increased radioactivity

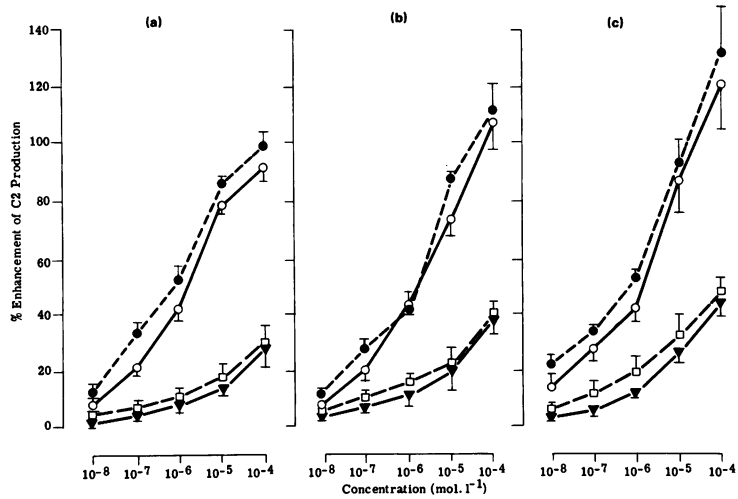

Fig. 2. Effect of adrenergic receptor antagonists (O–O) on the enhancement of C2 synthesis produced by (a) adrenaline, (b) noradrenaline and (c) phenylephrine. Propranolol (●–● 10^{-5} mol.l^{-1}) did not affect the enhancement produced by catecholamines, whereas phentolamine (□–□, 10^{-5} mol.l^{-1} and pentolamine + propranolol (▼–▼ both at 10^{-5} mol.l^{-1}) reversed the effect. C2 levels, expressed as percentage enhancement of untreated control cultures, were measured on day 7. Each point represents the mean (± s.e.m.) of three experiments performed in duplicate on different donors.

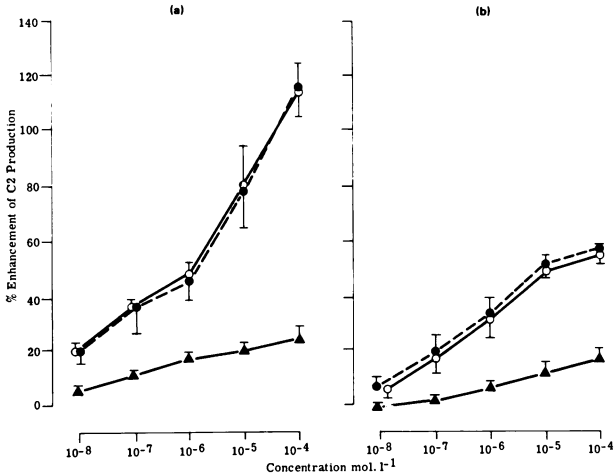

Fig. 3. (a) Effect of prazosin (▲–▲, 10^{-5} mol.l^{-1}) and yohimbine (●–●, 10^{-5} mol.l^{-1}) on the enhancement of C2 synthesis produced by phenylephrine (○–○). (b) Effect of prazosin (▲–▲, 10^{-5} mol.$l.^{-1}$) and yohimbine (●–●, 10^{-5} mol.l^{-1}) on the enhancement of C2 synthesis produced by clonidine (○–○). C2 levels, expressed as percentage of untreated control cultures, were measured on day 7. Each point represents the mean (± s.e.m.) of three experiments performed in duplicate.

precipitated by specific antibodies (Fig. 4). In all instances enhancement was prevented by phentolamine, but not by propranolol.

The proportion of specific complement protein (antibody precipitable counts) to total protein (acid precipitable counts) varied for each protein, being highest for properdin (1·1%) and lowest for β1H (0·5%) (Fig. 5). Although there was a slight fall in the ratio of antibody precipitable to total

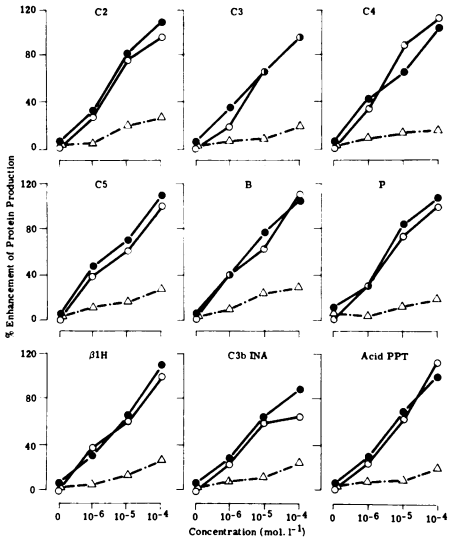

Fig. 4. Effect of phenylephrine (●–●) on the incorporation of ^{3}H-amino acids into acid precipitable protein (Acid ppt), and counts precipitated by antibodies to C2, C4, C3, C5, factor B (B), properdin (P) C3b inactivator (C3bINA) and β1H. Phentolamine (△–△, 10^{-5} mol.l^{-1}) abrogated the increased produced by phenylephrine whereas propranolol (○–○, 10^{-5} mol.l^{-1}) was ineffective. Incorporation measured on day 7. Each point represents the mean of two experiments performed in duplicate.

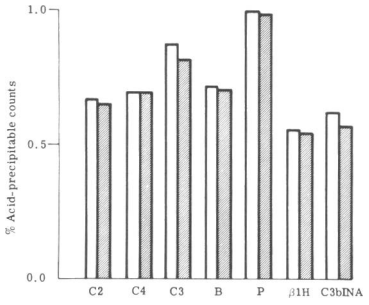

Fig. 5. Incorporation of ^{3}H-amino acids into complement proteins in control (□) and phenylephrine (10^{-4} mol.l^{-1}; ▨) treated cultures. Antibody precipitable counts are expressed as a percentage of acid precipitable counts (total protein synthesis).

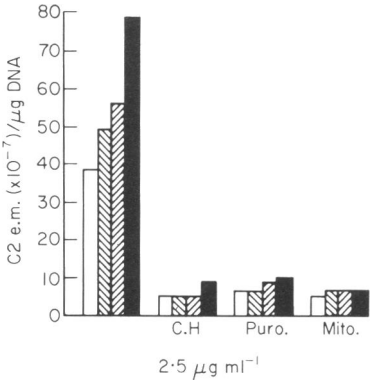

Fig. 6. Inhibition of C2 synthesis in control (□), adrenaline (▧), noradrenaline (▨) and phenylephrine (■) treated cultures, by cycloheximide (CH), puromycin (Puro) and mitomycin C (Mito) all at 2.5 μg/ml. The set of bars on the extreme left represent C2 levels in control cultures. The concentration of adrenergic agonists was 10^{-5} mol.l^{-1}. C2 levels were measured on day 7. Each point represents the mean of two experiments performed in duplicate on different donors.

protein in phenylephrine treated compared with control cultures this was not significant (Fig. 5).

Effect of protein synthesis inhibition on enhancement of C2 synthesis

The addition of cycloheximide (2·5 μg/ml), puromycin (2·5 μg/ml) or mitomycin C (2·5 μg/ml) to monocyte cultures inhibited synthesis in control and phenylephrine treated cultures (10^{-5} mol.l^{-1}) (Fig. 6). Removal of cycloheximide and puromycin, but not mitomycin C, on day 7 resulted in partial restoration of C2 synthesis.

DISCUSSION

Increased synthesis of complement components occurs in most diseases in which complement activation occurs (Ruddy *et al.*, 1975). Although the hepatocyte and the mononuclear phagocytes have been shown to be the cells which synthesize complement components (Colten, 1976; Whaley, 1980), the factors controlling their synthesis are as yet undefined.

In this paper we have presented evidence that the synthesis of complement components by human monocytes is increased by adrenergic agonists. The receptor mediating this effect must be an α_1 receptor, as adrenaline and noradrenaline, which both act at α and β receptors, and

phenylephrine a selective α receptor agonist increase synthesis of C2, whereas the β receptor agonist isoprenaline had no effect (Fig. 1). This conclusion was supported by the observation that phentolamine an α receptor antagonist, but not propranolol, a β receptor antagonist, abrogated the stimulatory enhancement of C2 production when adrenergic agonists were added to the tissue culture medium (Fig. 2). The ability of prazosin, rather than yohimbine to reverse the effect of phenylephrine (Fig. 3) indicates that the effect is mediated by α_1 receptors. Clonidine is considered to be an α_2 receptor agonist, but the enhancement of C2 production induced by this agent was abrogated by prazosin (Fig. 3), showing that clonidine has α_1 agonist effects also.

The experiments examining the incorporation of ^{3}H-amino acids into protein showed that synthesis of eight complement components was increased, as was total protein synthesis (Fig. 4). The ratio of complement protein synthesis to total protein synthesis was essentially the same in control and phenylephrine treated cultures (Fig. 5). One must conclude from these observations that the ability of monocytes to synthesize protein is probably enhanced by α adrenergic stimulation. That increased synthesis, rather than increased secretion of preformed protein occurs when α adrenergic agonists are added to monocyte cultures is shown by the inhibitory effects of cycloheximide, puromycin and mitomycin C (Fig. 6).

The failure of isoprenaline to affect C2 synthesis possibly indicates that monocytes do not express functionally active β adrenergic receptors. Alternatively, the metabolic consequences of β adrenergic receptor stimulation do not affect C2 synthesis. We have previously been shown that increased levels of cAMP inhibit the production of complement components whereas concentrations of cGMP do not appear to affect synthesis (Lappin & Whaley, 1981). As α adrenergic agonists inhibit adenylcyclase activity, thereby reducing cAMP levels (Rodbell, 1980) it is tempting to speculate that this reduction may be responsible for increased complement component synthesis. However in order to support this unusual suggestion, monocyte cAMP levels must be determined for increased complement component synthesis. Experiments designed to measure monocyte cAMP levels following α adrenergic stimulation are currently in progress.

In normal individuals, under resting conditions the mean serum levels of noradrenaline and adrenaline are $2{\cdot}5 \times 10^{-9}$ mol.l^{-1} and $0{\cdot}4 \times 10^{-9}$ mol.l^{-1} respectively (Cryer, 1980). Our studies have shown that only minimal enhancement of C2 synthesis is produced by catecholamine concentrations of 1×10^{-9} mol.l^{-1}. It is therefore unlikely that the synthesis of complement components by mononuclear phagocytes in inflammatory exudates is affected significantly by these hormones in the circulation. However, both the liver and spleen and richly supplied by post-ganglionic sympathetic nerve fibres (Forssman & Ito 1977; Nobin *et al.*, 1978). The possibility that catecholamines modulate the synthesis of complement by macrophages in these tissues requires investigation.

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