Cyclic AMP antagonizes mitogen-induced accumulation of inositol phosphates in human peripheral mononuclear leucocytes *in vitro*

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The effect of intracellular increases of cyclic adenosine monophosphate (cAMP) on the accumulation of inositol phosphates (IPs) in response to various mitogens *in vitro* in human peripheral mononuclear leucocytes (MNL) was investigated. The β -adrenoceptor agonists inhibited mitogen-induced IPs-accumulation by about 30–50% with a rank order of potency isoprenaline > adrenaline $\gg$ noradrenaline. This rank order corresponded with the order of potency of the β -adrenoceptor agonists to generate cAMP. The β -adrenoceptor agonist-induced inhibition of IPs-accumulation in response to mitogen could be completely prevented by the β_2 -adrenoceptor selective antagonist ICI 118,551, but not by the β_1 -adrenoceptor selective antagonist CGP 20712A. Furthermore, the isoprenaline-induced inhibition of IPs-accumulation in response to mitogen could be enhanced by the phosphodiesterase inhibitor IBMX. We conclude that cAMP antagonizes mitogen-induced IPs-accumulation in human peripheral MNL *in vitro*.

Keywords cyclic AMP inositol phosphates mononuclear leucocytes

Introduction

Activation of lymphocytes by mitogens is associated with an early increase in inositol phosphates and diacylglycerol (for reviews see Berridge & Irvine, 1984; Hadden, 1988) and the subsequent expression of interleukin 2 (IL-2) receptors and production of IL-2. Several studies have indicated that cAMP can inhibit lymphocyte proliferation (for review see Kammer, 1988) and also a β -adrenoceptor mediated inhibition of IL-2 receptor expression has been reported (Feldman *et al.*, 1987). However the mechanism is not known. As in many other systems the biological effects of cAMP are opposite to those of IPs it might be possible that the anti-proliferative effect of cAMP is due to inhibition of IPs formation. To test this hypothesis we investigated the effects of β -adrenoceptor agonists on the accumulation of [³H]-IPs in response to various mitogens *in vitro* in human peripheral MNL.

Methods

MNL were isolated from heparinized blood by the method of Böyum (1968).

Turnover of phosphatidylinositols, measured as the accumulation of [³H]-IPs was determined as described by Minneman & Johnson (1984). In short, MNL were incubated with [³H]-myo-inositol in Krebs-Henseleit solution for 2 h, washed, stimulated with various stimuli as indicated and incubated for another 30 min in the presence of 10 mM LiCl. Accumulated water-soluble [³H]-IPs were separated by extraction with a water-methanol/chloroform system and anion exchange chromatography. The mitogens phytohemagglutinin (PHA, Wellcome, Beckenham, U.K.), concanavalin A (Con A, Sigma, Deisenhofen, FRG) and pokeweed mitogen (PWM, Boehringer, Mannheim, FRG) were used as stimuli. The accumulation of cAMP in intact cells during a 15 min incubation

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period was measured as described by Brodde *et al.* (1985).

Results

Incubation of MNL with mitogens ($0.2\text{--}3.6\text{ }\mu\text{g PHA ml}^{-1}$; $2\text{--}36\text{ }\mu\text{g Con A ml}^{-1}$; $0.3\text{--}4.8\text{ }\mu\text{g PWM ml}^{-1}$) dose-dependently caused a 4 to 7-fold increase in the formation of $[^3\text{H}]\text{-IPs}$. Plateau values were reached for PHA and PWM at approximately $3\text{ }\mu\text{g ml}^{-1}$ and for Con A at approximately $30\text{ }\mu\text{g ml}^{-1}$; they were highest for Con A and lowest for PWM. For subsequent experiments, accumulations of $[^3\text{H}]\text{-IPs}$ by $1.8\text{ }\mu\text{g PHA ml}^{-1}$, $18\text{ }\mu\text{g Con A ml}^{-1}$ and $2.4\text{ }\mu\text{g PWM ml}^{-1}$ were assessed.

In the presence of the β -adrenoceptor agonists isoprenaline (ISO), adrenaline (A) and noradrenaline (NA), mitogen-induced $[^3\text{H}]\text{-IPs}$ accumulation was depressed by about 30 to 50%. The rank order of potency for the inhi-

bition of the mitogen response was: $\text{ISO} > \text{A} \gg \text{NA}$ (Table 1). This order of potency corresponded with the order of potency of the β -adrenoceptor agonists to generate cAMP (data not shown).

The β -adrenoceptor agonist-induced inhibition of the mitogen response could be completely blocked by $100\text{ nM ICI 118,551}$, but was not affected by 500 nM CGP 20712A .

On the other hand, the ISO-induced inhibition of the mitogen response was markedly enhanced by the phosphodiesterase inhibitor IBMX (0.1 mM), resulting in 70 to 90% inhibition of $[^3\text{H}]\text{-IPs}$ accumulation.

Discussion

In this study, β -adrenoceptor agonists were shown to inhibit mitogen-induced accumulation of IPs in human peripheral MNL. The β -adrenoceptor involved in this process, was of the β_2 subtype since the effect could be inhibited by the β_2 -adrenoceptor selective antagonist ICI 118,551 but not by the β_1 -adrenoceptor selective antagonist CGP 20712A. The finding that inhibition of the mitogen response could be enhanced by the phosphodiesterase inhibitor IBMX, suggests an important role for cAMP in this process.

In lymphocytes, production of diacylglycerol and inositol phosphates in response to mitogens, is regarded as an initial signal for lymphocyte proliferation. Thus the anti-proliferative effect of cAMP might be due to inhibition of this initial signal. Accordingly, in physiological and pathological conditions of elevated plasma catecholamine levels, enhanced activation of lymphocyte β_2 -adrenoceptors, leading to increased intracellular cAMP levels, might influence cellular immune function.

Table 1 EC_{50} values for β -adrenoceptor agonists inhibiting mitogen-induced $[^3\text{H}]\text{-inositol phosphates (IPs)}$ accumulation. Dose-response curves for the β -adrenoceptor agonists isoprenaline (ISO), adrenaline (A) and noradrenaline (NA) in inhibiting $[^3\text{H}]\text{-IPs}$ accumulation caused by $1.8\text{ }\mu\text{g PHA ml}^{-1}$, $18\text{ }\mu\text{g Con A ml}^{-1}$ and $2.4\text{ }\mu\text{g PWM ml}^{-1}$ were performed. Each value was calculated by non-linear regression from a curve consisting of mean values of three to ten experiments in duplicate

	PHA	Con A $\text{EC}_{50}\text{ (nM)}$	PWM
ISO	5.7	37	6.6
A	18	82	15
NA	560	990	560

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