RAPID INCREASE OF PHOSPHORIBOSYL PYROPHOSPHATE CONCENTRATION AFTER MITOGENIC STIMULATION OF LYMPHOCYTES

T. HOVI, A. C. ALLISON and J. ALLSOP

Clinical Research Centre, Watford Road, Harrow, HA1 3UJ Middlesex, England

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1. Introduction

The initiation of DNA-synthesis in lymphocytes by mitogens has been widely studied as a model for initiation of the proliferation in resting cells. Several different biochemical events have been described following shortly (within one hour) after exposure of lymphocytes to mitogens, but the possible causal relations of these events to the initiation of DNA synthesis are still poorly understood [1]. Phytohaemagglutinin (PHA) induces an increased influx of calcium ions through the plasma membrane into the cells within a few minutes [2,3], and calcium ionophore itself acts as a mitogen for lymphocytes [4–6]. Another early event is a considerable increase of the levels of cyclic guanosine 3′:5′ monophosphate (cGMP) in the cells, detected within minutes of exposure to PHA [7]. These two processes may be causally related since the enzyme by which cGMP is synthesized is activated by calcium in other cells [8] as well as in lymphocytes (our unpublished observations). Although activation of protein kinases has been generally considered to be the main mechanism by which cyclic purine nucleotides participate in the regulation of cellular metabolism [9], other important modes of action may exist. Indeed, the enzyme responsible for the synthesis of phosphoribosyl pyrophosphate (PRPP), PRPP synthetase, was recently reported to be allosterically regulated by cyclic purine nucleotides [10]. Cyclic cGMP was a potent stimulator of the enzyme, while cAMP competitively inhibited the stimulation by cGMP [10]. PRPP is a common precursor for both the de novo and the 'salvage' pathways of purine biosynthesis in mammalian cells [11,12]. PRPP concentration is considered to be one of the main regulators of the first enzyme in the novo pathway, glutamine-phosphoribosyl pyrophosphate amidotransferase [13], and thereby an important effector of the overall rate of purine biosynthesis.

Activation of purine biosynthesis could be the way by which mitogens initiate DNA synthesis in lymphocytes. This hypothesis is supported by our present data, which show that PHA induces a rapid increase in the concentration of PRPP in cultured human peripheral blood lymphocytes.

2. Materials and methods

2.1. *Cell cultures*

Human peripheral blood lymphocytes were purified from buffy coats of healthy persons by a modification of the Ficoll-Hypaque technique [14], as described in detail elsewhere [6]. The final cell suspensions, containing more than 95% trypan blue excluding mononuclear leucocytes, were incubated at 37°C in RPMI-1640 medium supplemented with 10% foetal calf serum. A small volume of PHA in phosphate buffered saline was added to a final concentration of 2 μg/ml, and the cells were incubated at a density of 5×10^6 cells/ml in a humidified atmosphere (5% v/v CO_2 in air).

2.2. *PRPP measurement*

To measure cellular PRPP pools $50-100 \times 10^6$ cells per sample were harvested by centrifugation, washed twice with phosphate buffered saline and frozen as a cell pack at −20°C until assayed. Immediately before the assay the cell pack was thawed, one volume of distilled water was added, and the cells were broken by ultrasonic treatment. The homo-

genates were then centrifuged for 15 min at 4000 *g* at 4°C. The supernatants were assayed for PRPP concentration enzymatically using either exogeneous hypoxanthine-guanine phosphoribosyl transferase (HGPRTase) [15] or the endogenous adenine phosphoribosyl transferase (APRTase) [16] and the corresponding ^{14}C-labelled cosubstrates, hypoxanthine or adenine. In both methods the reaction was stopped by addition of excess EDTA and cooling immediately to 0°C. The monophosphates were separated by high voltage electrophoresis in 0.02 M sodium lactate pH 3.6 at 7 kV for 25 min. The spots, co-electrophoresed with IMP or AMP, were located by u.v. light, cut out and counted for radioactivity in a toluene-based scintillation fluid with an efficiency of about 75%.

2.3. *Sources and special properties of reagents used*

The buffy coats were kindly provided by the North London Blood Transfusion Centre, Edgware, Middlesex Purified phytohaemagglutinin type HA 16 was from Wellcome Reagents Limited, Beckenham, UK. RPMI-1640 and foetal calf serum were supplied by GIBCO-BioCult, Paisley, Scotland. [^{14}C]adenine and [^{14}C] hypoxanthine were from the Radiochemical Centre, Amersham, UK. HGPRTase was purified from human erythrocytes according to the method of Krenitsky et al. [17]. Other chemicals were from Sigma Chemical Co.

3. Results

The low level of PRPP in unstimulated human peripheral blood lymphocytes did not change significantly during the observation period. Adding 2 μg/ml of PHA to the cell cultures caused a rapid increase in the level of PRPP, detectable after 5 min and reaching a maximum at 15 to 20 min (fig.1). Later on the concentration of PRPP decreased to the level of the unstimulated cells. This type of rapid and transient increase was observed in all four experiments carried out with duplicate samples. In one experiment, however, the level of PRPP was three-fold that of the control cultures at 15 min and was still elevated at 60 min. Three of these experiments were carried out using the endogenous APRTase enzyme and one using exogeneous HGPRTase. Both methods gave a similar pattern of the PHA-induced increase of PRPP level, although the absolute values of PRPP concentration observed using the endogenous APRTase were slightly higher than those obtained by the exogeneous HGPRTase method.

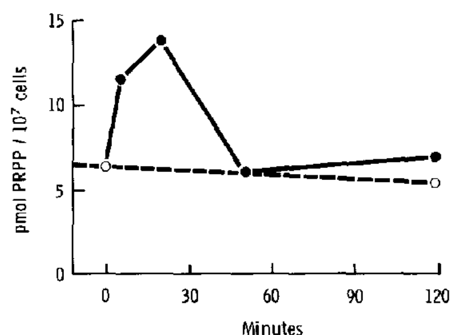

Fig.1. Time course of the PHA-induced increase in PRPP concentration in human peripheral blood lymphocytes. HPB lymphocytes were cultured with 2 μg/ml PHA at 37°C. Aliquots of 6 × 10^7 cells were harvested at the times indicated and assayed for PRPP by using the endogenous APRTase enzyme (see Material and methods) (○–○) Non-stimulated cells; (●–●) PHA-treated cells. Mean of duplicate samples is given.

4. Discussion

We have shown that exposure of human peripheral blood lymphocytes to phytohaemagglutinin at the optimal mitogenic concentration causes a rapid increase of the cellular levels of phosphoribosylpyrophosphate, a precursor and regulator of the novo pathway of purine biosynthesis. It is important to note that the observed time-course in the PHA-induced increase of PRPP concentration is similar to that reported for the PHA-induced change in the level of cGMP in human lymphocytes [7]. Thus, our results suggest that cGMP may also stimulate PRPP synthetase in intact lymphocytes. Increased PRPP level in mitogen-stimulated lymphocytes may activate the biosynthesis of purine nucleotides, an event which may be a necessary prerequisite for the initiation of a new cell cycle. The fact that the observed increase of PRPP concentration decayed rapidly may be a reflection of secondarily increased utilization of PRPP An alternative explanation is that only a pulse of

PRPP and purine biosynthesis is needed to push a resting lymphocyte from Go to G1.

The relative importance of the de novo pathway of purine biosynthesis and interconversions of purine nucleotides for the proliferation of lymphocytes is pinpointed by the well known value of purine derivatives as immunosuppressants and in anti-leukaemia chemotherapy [12], and by the fact that a congenital lack of adenosine deaminase leads to a severe combined immunodeficiency [18]. While this work was in progress Chambers et al. reported Concanavalin A-induced activation of the de novo pathway of purine biosynthesis in mouse spleen cell cultures, detected 8 hr after adding the mitogen [19], and increased PRPP levels have been observed in human peripheral blood lymphocytes incubated for 3 days with PHA [20]. Our work is the first in which increased PRPP concentration has been associated with the early events of the mitogenic response of lymphocytes. Whether this change results in an early activation of purine biosynthesis in mitogen-stimulated lymphosytes, remains to be shown by further studies.

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