Volume 318, number 3, 249–252 FEBS 12173 March 1993

Effect of pteridine derivatives on intracellular calcium concentration in human monocytic cells

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Received 11 January 1993

Pteridines are heterocyclic compounds which are synthesized and released by human monocytes/macrophages following stimulation by interferon-γ. Their concentration in various body fluids proved to be indicative for the stimulation of the cellular immune system, and determination of pteridines has become an important diagnostic tool. We show that pteridine derivatives, namely neopterin (N), 7,8-dihydroneopterin (NH_2), and 5,6,7,8-tetrahydrobiopterin (BH_4) increase intracellular calcium (Ca_i) in human monocytic cells. Significant increases of Ca_i are observed at 10 nmol/l NH_2, at 100 nmol/l BH_4 and at 1 mol/l N, i.e. at concentrations encountered in vivo. At a concentration of 1 μmol/l, Ca_i is increased (from a control value of 145 ± 7 nmol/l) to 464 ± 62 nmol/l (NH_2), 340 ± 41 nmol/l (BH_4) and 344 ± 46 nmol/l (N), respectively. The increase of Ca_i depends on the presence of extracellular calcium and is likely to be due to activation of a calcium channel. We show that the absence of extracellular calcium or the addition of lanthanum ions to the extracellular fluid fully reverses the pteridine-induced increase of Ca_i. According to these observations, pteridines may mimick the effects of other inflammatory mediators on monocytic cells and seem to be involved in the crosstalk of immunocompetent cells.

Intracellular calcium; Macrophage; Neopterin; Tetrahydrobiopterin; Lanthanum

1. INTRODUCTION

Pteridines are pyrazino-pyrimidine compounds which are synthesized by the cleavage of guanosine triphosphate (GTP) catalyzed by the enzyme GTP-cyclohydrolase I [1]. Depending on the enzyme pattern of the cell the first intermediate of the biosynthetic pathway, 7,8-dihydroneopterin-triphosphate, is either converted to 7,8-dihydroneopterin and neopterin or to 5,6,7,8-tetrahydrobiopterin [2], the latter known to be an essential cofactor for hydroxylation reactions [3] or nitric oxide synthase [4,5]. Neopterin and 7,8-dihydroneopterin are produced in excess by human macrophages/monocytes upon activation by interferon-γ [6]. The diagnostic value of neopterin has been established in autoimmune diseases, rejection of transplanted organs, malignant diseases, infectious diseases such as AIDS or hepatitis (reviewed in [7]). While it was evident that stimulation of the cellular immune system leads to an increase of pteridine biosynthesis and release, a possible function of pteridines in the inflammatory process remained elusive. The present study has been performed to identify cellular effects of pteridines in human monocytic cells with characteristics of macrophages (THP-1) [8]. Since an increase of intracellular calcium concentration was discussed to play a role in the activation of macrophages [9,10], the effect of pteridines on intracellular calcium activity in THP-1 cells was tested for. To this end, fura-2 fluorescence was determined in monocytic cells before and during application of neopterin, dihydroneopterin and tetrahydrobiopterin. As a result, all three pteridines were able to enhance intracellular calcium activity at concentrations encountered in vivo.

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2. MATERIALS AND METHODS

Experiments were performed with the human myelomonocytic cell line THP-1 [8] obtained from the American Type Culture Collection (Rockville, MD). Cells were grown in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS), 2 mmol/l glutamine, 100 U/ml penicillin and 0.1 ng/ml streptomycin. Cells were seeded at a density of 1×10^6/ml and cultured in serum free medium for 24 h. Then serum was added (final concentration: 10% FCS) and effects of pteridines on the intracellular calcium concentration were investigated between 1–8 h after serum supplementation.

Determinations of intracellular calcium activity were made utilizing fura-2 fluorescence. To this end the cells were incubated for 15 min with 2.5 μmol/l fura-2-AM (Molecular Probes, Junction City, OR, USA), thereafter fixed on polylysine-coated cover glasses and mounted in a perfusion chamber, enabling determination of fluorescence signals at a single cell level. Measurements were made under an inverted phase-contrast microscope (IM-35, Zeiss, Germany) equipped for epifluorescence and photometry (Hamamatsu, Herrsching, Germany) [11]. Light from a xenon arc lamp (XB075, Osram, Berlin, Germany) was directed through a grey filter (nominal transmission 3.16% Oriel, Darmstadt, Germany), alternatively through a 340-nm and 380-nm, respectively, interference filter (halfwidth 10 nm, Oriel, Darmstadt, Germany) and a diaphragm and was deflected by

a dichroic mirror (FT425, Zeiss, Germany) into the objective (Plan-Neofluar 63× oil immersion, Zeiss, Germany). The emitted fluorescence was directed through a 420 nm cutoff filter to a photomultiplier tube (R4829, Hamamatsu, Herrsching, Germany). To reduce the region from which fluorescence was collected a pinhole was placed in the image plane of the phototubus (limitation to a circular area of 60 μm diameter).

Fluorescence values were corrected for cellular autofluorescence; intracellular calcium (Ca_i) was calculated from the ratio (r) of the fluorescence intensities at the two different excitation wave lengths (340 nm/380 nm) [12,13]:

$$Ca_i = K_d \, Sf_2 \, (R - R_{min})/Sb_2 \, (R_{max} - R)$$

where R_{min} and R_{max} are the fluorescence ratios corrected for autofluorescence at experimental condition, at minimal and maximal calcium binding. K_d is the dissociation constant for fura-2 (= 225), Sf_2 and Sb_2 are the proportionality coefficients for the fluorescence at 380 nm excitation of free and calcium-bound dye, respectively. R_{min} and Sf_2 were determined by exposure of the cells to 2 mmol/l Mn^{2+} + 20 μmol/l A 23187, R_{max} and Sb_2 after exposure of the cells to either, 20 μmol/l digitonin (Sigma, Munich, Germany) or 20 μmol/l A 23187.

The extracellular perfusate was composed of (all numbers mmol/l): 114 NaCl, 5.4 KCl, 0.8 $MgCl_2$, 1.2 $CaCl_2$, 0.8 Na_2HPO_4, 0.2 NaH_2PO_4, 20 $NaHCO_3$ and 5.5 glucose. The solution was equilibrated with 5% carbon dioxide and 95% air (pH 7.4), and experiments were carried out at 37°C.

Applicable data are expressed as arithmetic means ± S.E.M. Statistical analysis was made by paired or unpaired t-test, where applicable. Statistically significant differences were assumed at $P < 0.05$.

Table I

Intracellular calcium concentration in nmol/l (Ca_i) in the absence of pteridines (control), in the absence of extracellular calcium (0 Ca^{2+}), in the presence of 100 μmol/l lanthanum ions (La^{3+}), and in the presence of various concentrations of neopterin (N), 7,8-dihydroneopterin (NH_2), 5,6,7,8-tetrahydrobiopterin (BH_4) (n = number of experiments)

Condition	Ca_i	(n)
Control	145 ± 7	40
10 nmol/l N	210 ± 86	4
100 nmol/l N	251 ± 82	4
1 μmol/l N	344 ± 46	5
10 μmol/l N	425 ± 90	4
10 nmol/l NH_2	254 ± 51	6
100 nmol/l NH_2	249 ± 48	6
1 μmol/l NH_2	464 ± 62	8
10 μmol/l NH_2	553 ± 74	6
10 nmol/l BH_4	183 ± 24	5
100 nmol/l BH_4	249 ± 35	8
1 μmol/l BH_4	340 ± 41	9
10 μmol/l BH_4	346 ± 23	5
0 Ca^{2+}	104 ± 10	13
0 Ca^{2+} + 1 μmol/l NH_2	102 ± 11	13
La^{3+}	144 ± 15	4
La^{3+} + 1 μmol/l NH_2	138 ± 13	4

3. RESULTS AND DISCUSSION

As listed in Table I, intracellular calcium activity approaches 145 nmol/l in the absence of pteridines, i.e. a value within the range reported previously for human monocytic cells [14]. The pteridines employed in this study, i.e. neopterin (N), dihydroneopterin (NH_2), and tetrahydrobiopterin (BH_4) lead to a rapid, transient increase of intracellular calcium (Fig. 1). The most effective pteridine is NH_2, followed by BH_4 and N. The concentrations required to obtain significant effects are 10 nmol/l for NH_2, 100 nmol/l for BH_4 and 1 μmol/l for N. Such neopterin concentrations are approaches in blood during activation of the immune system [7,15,16], and local concentrations might even be higher. Accordingly, the effects of the pteridines on the intracellular calcium concentration of monocytic cells may also be expected to occur in vivo following activation of the

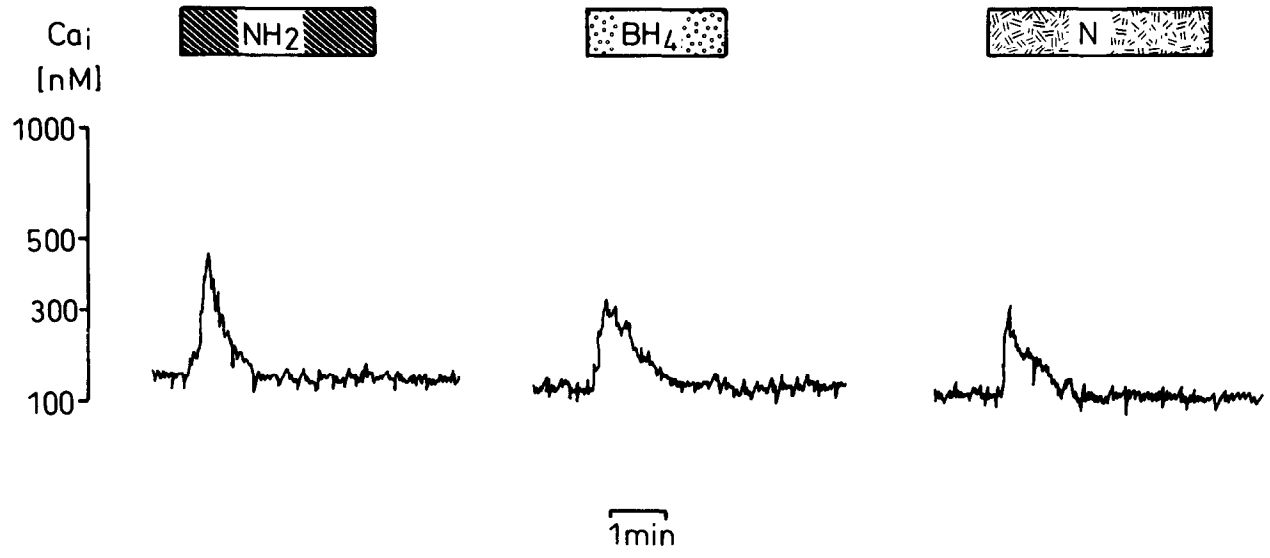

Fig. 1. Effect of pteridine derivatives on intracellular calcium concentration (Ca_i) in human monocytic cells. Original tracings showing the effect of 7,8-dihydroneopterin (NH_2), 5,6,7,8-tetrahydrobiopterin (BH_4), and neopterin (N) (final concentrations 1 μmol/l each).

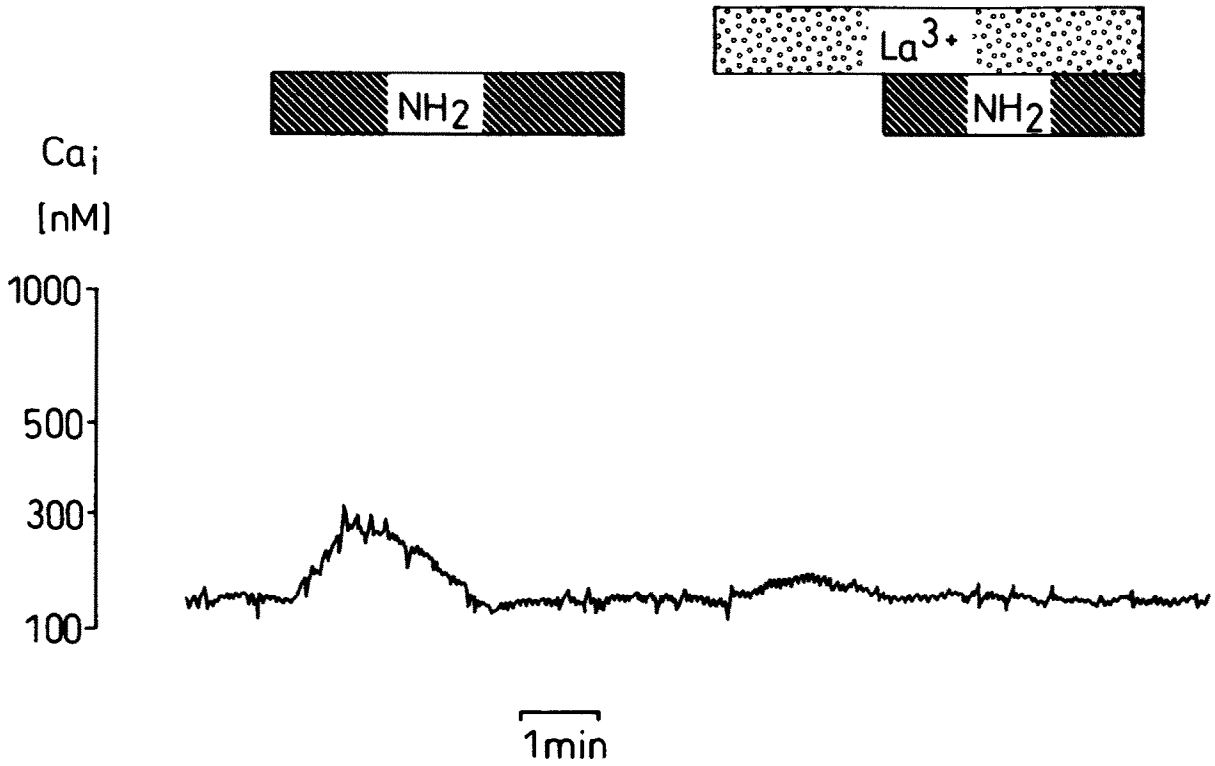

Fig. 2. Effect of lanthanum ions (La^{3+}, 100 μmol/l) on pteridine induced increase of intracellular calcium concentration (Ca_i). Original tracings, showing the effect of 1 μmol/l 7,8-dihydroneopterin (NH_2) on Ca_i in both the presence and absence of lanthanum ions.

macrophages by interferon-γ. The effect of the pteridines is variable, most probably depending on the cell cycle and/or differentiation stage of the cells. When cells are synchronized by serum deprivation and then stimulated by the addition of FCS, the pteridine effects on Ca_i are most pronounced in the G_1 phase when cells are also differentiated badly (not shown). This is in accordance with recent findings demonstrating that calcium effects in THP-1 monocytic cells depend on the differentiation stage of the cell [17]. As shown for NH_2 (Table I), the pteridine effect on the intracellular calcium concentration requires the presence of extracellular calcium. Deprivation of extracellular calcium by the addition of EGTA (1 mmol/l) decreases Ca_i to some 104 nmol/l. In none of the experiments performed NH_2 (1 μmol/l final concentration) was able to induce an increase of Ca_i under these conditions (Table I). Thus, the presence of a pteridine-inducible calcium channel seems to be evident since the addition of lanthanum ions (100 μmol/l), known to block calcium channels [18], abolishes the pteridine-induced increase of Ca_i (Table I, Fig. 2). A number of mediators of the immune system have been reported to increase the intracellular calcium concentration, such as interferon-γ [14,19], the cytokine-stimulating pteridine biosynthesis in human monocytes/macrophages [6]. An increase of intracellular calcium has in turn been shown to play an important role in the activation of human monocytic cells [9,10,14,19,20]. Accordingly, the ability of pteridine derivatives to increase the intracellular calcium concentration in human monocytic cells indicates that these macrophage-derived substances may be involved in the regulation of calcium-mediated macrophage activation.

Acknowledgements: Financial support by the Austrian Research Fund 'Zur Förderung der wissenschaftlichen Forschung', Project FFWF 9257, is gratefully acknowledged.

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