

# Influence of nonselective and selective phosphodiesterase inhibitors on cAMP levels in lymphocytes after a single administration in mice

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## Abstract

The cyclic adenosine 3',5'-monophosphate (cAMP) is an important factor that modulates the activity of immune cells, including lymphocytes. The PDE inhibitors were administered once: aminophylline, a nonselective PDE inhibitor, at a dose of 20 mg/kg i.m., milrinone, a selective PDE3 inhibitor, at a dose of 1 mg/kg i.m., and sildenafil, selective PDE5 inhibitor, at a dose of 1 mg/kg p.o.

The purpose of this study was to determine the effect of the PDE inhibitors under investigation of cAMP levels in the lysates of lymphocytes obtained from thymus, spleen and mesenteric lymph nodes. It was found that aminophylline, milrinone or sildenafil did not change the cAMP levels in thymocyte lysates. These drugs induced an increase in the levels of cAMP in the lysates of splenocytes. Administration of aminophylline did not influence the cAMP levels in the lysates of mesenteric lymph node lymphocytes. However, a decrease in the cAMP concentration in mesenteric lymph node cells was observed after a single administration of the selective PDE inhibitors: milrinone or sildenafil.

Aminophylline, milrinone and sildenafil had no impact on the cAMP level in lymphocytes from thymus. However, the drugs under investigation changed the cAMP content in lymphocytes from peripheral lymphatic organs. These effects depended on the type of a drug and its selectivity for PDE isozymes.

**Key words:** cAMP level, lymphocytes, aminophylline, milrinone, sildenafil, mice.

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## Introduction

The cyclic adenosine 3',5'-monophosphate (cAMP) as a second messenger, plays a key role in modulation of activity of numerous cells, including the cells of the immune system.

The cAMP is generated by transmembrane or soluble adenylyl cyclase in response to various extracellular stimuli [1, 2]. The only route of cAMP degradation is hydrolysis by cAMP phosphodiesterases (PDEs) [3, 4]. Currently, the PDE superfamily includes 11 different PDE families (PDE1-PDE11), each with several isoforms [5, 6]. PDE families differ, among others, in substrate specificity (cAMP

and/or cGMP). cAMP-specific families are PDE4, PDE7 and PDE8; cGMP-specific families are PDE5, PDE6 and PDE9; other families: PDE1, PDE2, PDE3, PDE10 and PDE11 hydrolyze both cyclic nucleotides [5].

The cAMP is strongly involved in the regulation of lymphocyte functions such as T cell activation. It regulates T lymphocyte activities through protein kinase A (PKA). PKA type I, predominant PKA isoform in T cells, is anchored to the T-cell receptor (TCR) [7, 8]. Stimulation of TCR alone, without CD28 costimulation, is followed by induction of cAMP-PKA pathway, resulting in inhibition of T cell activation [8-11]. TCR/CD28 costimulation induces the recruitment of PDE4/ $\beta$ -arrestin com-

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plex and leads to decrease of local cAMP concentration by PDE4 [8-12].

Thus, currently among PDE isozymes, PDE4 is a major target for the drugs modulating the activity of immune cells, including T lymphocytes [4, 10, 12-14]. These extensive studies of selective PDE4 inhibitors result in clinical applications of these drugs, e.g. in the treatment of chronic obstructive pulmonary disease, asthma or rheumatoid arthritis [4, 10, 12-14].

However, it was found that PDE3 also shows high activity in human T lymphocytes as PDE4. Moreover, the activity of PDE1, PDE2, PDE5, PDE7, and PDE8 was also detected in these cells [9, 10, 15, 16]. The PDE patterns did not differ in CD4<sup>+</sup> and CD8<sup>+</sup> human T cells from peripheral blood [16].

Predominant PDE in human B lymphocytes from peripheral blood is cytosolic PDE4. In the soluble fraction, PDE7 activity has also been found. Only marginal activity of PDE3 was detected in the particulate fraction of B lymphocytes. There were no activity of PDE1, PDE2 and PDE5 in B cells [17].

In our previous study conducted *in vivo*, it was shown that a single dose of aminophylline (nonselective PDE inhibitor), milrinone (selective PDE3 inhibitor), or sildenafil (selective PDE5 inhibitor), exerts modulatory effects on the lymphocyte subsets and Th1/Th2 cytokine production [18, 19].

The aim of the present investigation was to determine if aminophylline, milrinone or sildenafil, administered *in vivo*, change the cAMP levels in the lysates of lymphocytes from thymus, spleen or mesenteric lymph nodes. The drugs were administered once, at the same dose as in the previous studies.

## Material and methods

### Animals

The studies were conducted on 8-week-old female Balb/c mice, each weighing 18-22 g. Experimental animals were obtained from the Breeding Center of Laboratory Animals of the Institute of Occupational Medicine, Łódź, Poland. The principles of laboratory animal care (NIH publication No 86-23, revised 1985), and national laws regarding the protection of animals were observed. The study protocol was approved by the Local Ethics Committee in Wrocław, Poland (No 74/2008).

### Drugs and treatment

The PDE inhibitors were administered once at the therapeutic doses: aminophylline (Aminophyllinum, Pliva Kraków, Poland) at a dose of 20 mg/kg *i.m.*, milrinone (Corotrope, Sanofi-Synthelabo, Paris, France) at a dose of 1 mg/kg *i.m.* and sildenafil (Pfizer, Sandwich, UK) at a dose of 1 mg/kg *p.o.*

Parallely, mice in the control group received phosphate buffered saline solution – PBS (Institute of Immunology and Experimental Therapy, Wrocław, Poland), instead of the drugs. The volume of each dose was 0.1 ml per animal. Each experimental group consisted of 7 mice.

### Measurements

The mice were anesthetized with halothane (Narcotan, Zentiva, Prague, Czech Republic), 12 h after the drug administration. Thymuses, spleens and mesenteric lymph nodes were removed and placed in sterile, ice-cold PBS. The suspended cells were released from the lymphatic organs by passage through a nylon mesh. Then the cells were centrifuged (3000 g, 15 min, 4°C) on a layer of Ficoll 400 (Sigma)/Urografin 76% (Bayer Schering Pharma AG, Berlin, Germany) in a 1 : 3 ratio at a density of 1.076. After centrifugation, the cells were collected from the interphase and washed three times in cold PBS. After first wash erythrocytes in the suspensions of splenocytes were lysed using 0.84% ammonium chloride at 37°C for 5 min. After being washed, the cells were resuspended in PBS to a concentration of  $1 \times 10^7$  cells/ml. A commercial Parameter™ Cyclic AMP Immunoassay (R&D Systems, Inc., Minneapolis, USA, lot: 256958) was used to determine the levels of cAMP (pmol/ml) in the lysates of lymphocytes, according to the manufacturer's instructions. The absorbance was read at 450 nm, with the correction wavelength at 540 nm.

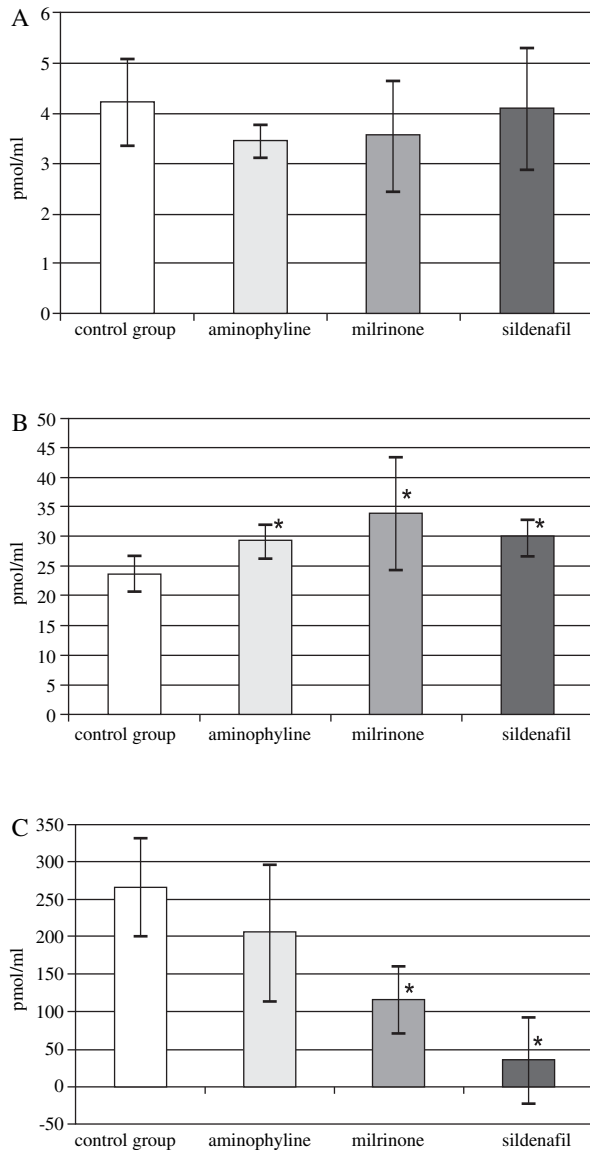
### Statistical analysis

The data obtained in this study were analyzed statistically using Student's *t*-test. The differences were considered significant at  $p < 0.05$ . The data were analyzed with Statistica 9.0 software.

## Results and discussion

The cAMP is an important factor that regulates the maturation of lymphocyte in thymus, inducing thymocyte apoptosis [20-22]. Therefore, PDE inhibitors can increase programmed cell death in thymus by the elevation of cAMP concentration [21].

Lalli *et al.* [21] reported that the activation of cAMP pathway in thymus led to a decrease in the number of double-positive (CD4<sup>+</sup>CD8<sup>+</sup>) cells. This effect was induced by various compounds that in different ways elevate cAMP levels, including 3-isobutyl-1-methyl-xanthine (IBMX), a nonselective PDE inhibitor. Therefore, the activation of cAMP pathway in thymus caused the block of thymocyte differentiation by increasing apoptosis, beyond the double-negative (CD4<sup>-</sup>CD8<sup>-</sup>) stage [21]. The data obtained in the present study show that a single dose of aminophylline, milrinone or sildenafil did not change the levels of cAMP in the thymocyte lysates, 12 h after administration of the drugs (Fig. 1). These results are in accordance with the data from our previous study, which showed that 12 h after a single



**Fig. 1.** The effect of a single dose of aminophylline, milrinone or sildenafil on the cAMP levels in lymphocytes from thymus (A), spleen (B) and mesenteric lymph nodes (C). The mean value ( $n = 7$ ) and standard deviation; \* $p < 0.05$  as compared to the control group

administration of these PDE inhibitors, an increase in the percentage of double-positive (CD4+CD8+) thymocytes was observed [18].

Most of the studies on the effects of the drugs or other compounds (e.g. cAMP analogs) on thymocyte maturation were conducted *in vitro* [20-22]. In the studies conducted *in vivo*, the extent to which the drugs are able to penetrate the tissues is very important. However, there are no data about the ability of the PDE inhibitors under investigation to cross the blood-thymus barrier.

In the present experiment, the changes in cAMP levels in lymphocytes from the peripheral lymphatic organs (spleen and mesenteric lymph nodes) were noted. The investigated PDE inhibitors induced an increase in the levels of cAMP in the splenocyte lysates (Fig. 1). On the other hand, the results obtained in the previous study showed that aminophylline, milrinone or sildenafil administered once did not change the subsets of splenocytes 12 h after drug administration [18].

Administration of aminophylline did not influence the cAMP levels in the lysates of mesenteric lymph node lymphocytes. Interestingly, a decrease in the cAMP concentration was observed after a single administration of the selective PDE inhibitors: milrinone or sildenafil (Fig. 1). The results of the previous experiment showed that a single dose of aminophylline or milrinone changed subsets of mesenteric lymph node cells, as manifested by an increased percentage of B cells (CD19+), and decreased percentage of T cells (CD3+), 12 h after administration. This effect was not observed at the same time point after sildenafil administration [18].

The differences between the effects of PDE inhibitors on lymphocyte subsets in different organs could result from both, direct impact, via cAMP level, and indirect impact, e.g. via cytokine production. It was previously found that the investigated drugs exerted a different effect on Th1/Th2 cytokine production. A single dose of aminophylline decreased IL-2 production 12 h after administration, while an increase in IL-5 production was observed 12 h after sildenafil administration. Milrinone did not change the Th1/Th2 cytokine synthesis [19].

The *in vitro* studies on the effects of PDE inhibitors on cAMP content in lymphocytes carried out by other authors gave different results. For example, Sheth *et al.* [23] reported that milrinone inhibited the cAMP hydrolysis in lymphocytes by 70%. In contrast, Giembycz *et al.* [15] observed that SK&F 95654 (selective PDE3 inhibitor) at a concentration range from 100 nM to 100  $\mu$ M did not increase cAMP levels in T cells.

In summary, the results of the present study show that the investigated PDE inhibitors have different effects on cAMP concentrations in lymphocytes from various lymphatic organs. These effects depend on the type of the drug and its selectivity for PDE isozymes. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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