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## Hypoxanthine Effects on Cyclic AMP Levels in Human Lymphocytes

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## Hypoxanthine Effects on Cyclic AMP Levels in Human Lymphocytes

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### ABSTRACT

We have measured hypoxanthine effect on cAMP levels in PBL in basal conditions (no agonist), and with the addition of 2-(p- [2-carboxyethyl] phenylethylamino)-5'-N-ethylcarboxamidoadenosine (CGS-21680, a specific A<sub>2</sub> receptor agonist). We have found that hypoxanthine, at 25  $\mu$ M and 50  $\mu$ M concentrations, increases cAMP levels in PBL in basal and A<sub>2</sub> agonist stimulated conditions.

*Key Words:* Hypoxanthine; Lesch-Nyhan; HPRT; Adenosine receptor.

### INTRODUCTION

We have reported that hypoxanthine excess causes inhibition of adenosine transport in peripheral blood lymphocytes (PBL). Decrease adenosine transport could lead to accumulation of extra-cellular adenosine that binds to adenosine A<sub>2A</sub> receptors and increases intra cellular cAMP production. We have tested this hypothesis by measuring hypoxanthine effect on cAMP levels in PBL.

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**Table 1.** Effect of hypoxanthine on cAMP levels in PBL.

Hypoxanthine ( $\mu\text{M}$ )	cAMP (fmol/ $10^6$ cells)	p vs. 5 $\mu\text{M}$	p vs. 0 $\mu\text{M}$
<i>BASAL</i>			
5	15 $\pm$ 5		
25	60 $\pm$ 10	P < 0.005	
50	74 $\pm$ 10	P < 0.005	
<i>+ CGS 21680 5 <math>\mu\text{M}</math></i>			
5	601 $\pm$ 11		
25	705 $\pm$ 10	P < 0.001	
50	723 $\pm$ 10	P < 0.001	
<i>+ CGS 21680 25 <math>\mu\text{M}</math></i>			
0	630 $\pm$ 24		
5	669 $\pm$ 32		NS
25	970 $\pm$ 27	P < 0.005	P < 0.0001

## METHODS

Hypoxanthine effect on cAMP levels in PBL were tested: a) in basal conditions (no agonist) or b) with the addition of 2-(p- [2-carboxyethyl] phenylethylamino)-5'-N-ethylcarboxamidoadenosine (CGS-21680, a specific A<sub>2</sub> receptor agonist) at 5  $\mu\text{M}$  and 25  $\mu\text{M}$  concentrations. Different hypoxanthine concentrations (0, 5, 25 or 50  $\mu\text{M}$ ) were added and cells incubated for 60 min at 37°C. Quantification of cAMP levels was determined using a commercially available enzyme-immunoassay kit (cAMP Biotrack, Amersham Biosciences).

## RESULTS

Results are shown in Table 1. Basal cAMP levels were significantly increased with the addition of 25  $\mu\text{M}$  and 50  $\mu\text{M}$  hypoxanthine versus 5  $\mu\text{M}$  hypoxanthine. cAMP levels in PBL stimulated with 5  $\mu\text{M}$  CGS 21680 were significantly increased with the addition of 25  $\mu\text{M}$  and 50  $\mu\text{M}$  hypoxanthine versus 5  $\mu\text{M}$  hypoxanthine. In PBL stimulated with 25  $\mu\text{M}$  CGS 21680, no significant differences were found between cAMP levels with the addition of 5  $\mu\text{M}$  hypoxanthine, or without hypoxanthine. However, 25  $\mu\text{M}$  hypoxanthine caused a significant increase in cAMP levels compared with basal or 5  $\mu\text{M}$  hypoxanthine concentrations.

## CONCLUSIONS

Hypoxanthine, at 25  $\mu\text{M}$  and 50  $\mu\text{M}$  concentrations, increases cAMP levels in PBL in basal and A<sub>2</sub> agonist stimulated conditions. Thus, increased hypoxanthine levels as excreted by HPRT deficient cells,<sup>[1,2]</sup> may decrease adenosine transport and lead to accumulation of extra-cellular adenosine that binds to adenosine A<sub>2A</sub> receptors and increases cAMP production.

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