INHIBITION OF HUMAN ERYTHROCYTE CASEIN KINASE BY METHYLXANTHINES

Study of inhibition mechanism

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1. Introduction

Methylxanthines interact with the cyclic nucleotide-dependent phosphorylation of proteins; they are potent inhibitors of cyclic nucleotide phosphodiesterase from human and animal tissues [1]: such that they increase cAMP level and indirectly stimulate cAMP-dependent phosphorylation.

In studies on phosphorylation of the red blood cell proteins we observed that methylxanthine derivatives inhibited cAMP-independent phosphorylation. Here, we show that two methylxanthine derivatives, caffeine and pentoxifylline (3,7-dimethyl 1-(5-oxohexyl) xanthine) are inhibitors of a purified casein kinase from human red cell and that they act as competitive inhibitors for the ATP binding site of enzyme.

2. Materials and methods

2.1. *Chemicals and enzymes*

Unlabelled ATP was obtained from Boehringer-Mannheim, [γ-^{32}P]ATP (spec. act. 3–4 Ci/mmol) from Radiochemical Centre, Amersham and partially dephosphorylated casein from Sigma. Pentoxifylline was supplied by Hoechst. Caffeine and other reagents were purchased from Merck. The casein kinase was prepared from human erythrocyte and purified as in [2]. Casein was used as substrate.

2.2. *Assay for human erythrocyte casein kinase*

For assay of casein kinase activity, the reaction mixture (100 μl) contained 0.05 M sodium acetate buffer (pH 6.5), 30 mM magnesium acetate, 125 mM KCl, 0.3 mM EGTA and substrate concentrations as indicated. The reaction was initiated by adding 5 μl enzyme (10 μg/ml). Incubations were done for 10 min at 30°C. The reactions were stopped by precipitation of the proteins with 4 ml ice-cold trichloracetic acid (5%). After centrifugation, the pellet was dissolved in 0.2 ml N NaOH, precipitated and dissolved twice again, then mixed with 10 ml scintillation fluid. Incorporated radioactivity was determined by liquid scintillation spectrometry (Intertechnic ABAC SL 400). Results of phosphorylation assay were expressed as pmol ^{32}P transferred from [γ-^{32}P]ATP/200 μg of casein in 10 min.

3. Results

3.1. *Effect of caffeine and pentoxifylline on casein kinase activity*

The effects of different concentrations of methyl-

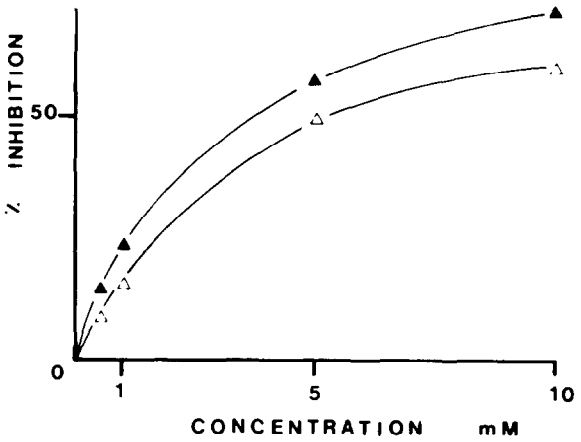

Fig.1. Effects of different concentrations of methylxanthines on casein kinase activity: ATP, 2.5 μM; casein, 2 mg/ml, (▲) caffeine; (△) pentoxifylline.

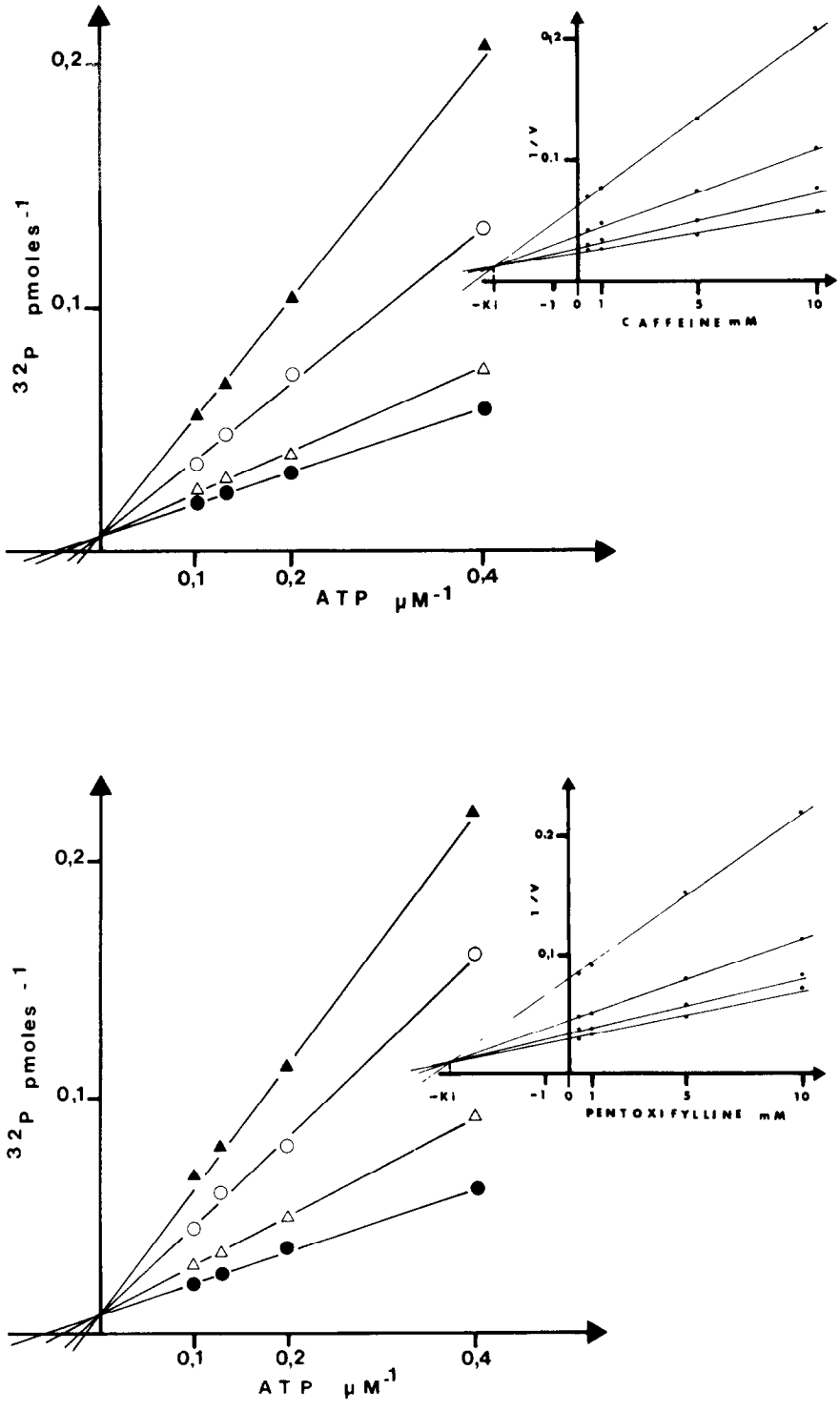

Fig.2. Lineweaver-Burk plot of initial rate as function of ATP concentrations: Casein. 2 mg/ml: caffeine (A) and pentoxifylline (B) at: (●——●) none; (△——△) 1 mM; (○——○) 5 mM; (▲——▲) 10 mM. The inserts show plots of reciprocal of initial velocity as function of ATP concentrations versus the concentration of methylxanthines.

xanthines on the casein kinase activity were studied under the above conditions with 2 mg casein/ml and 2.5 μM ATP.

The results are shown in fig.1. Caffeine and pentoxifylline were efficient inhibitors. At 10 mM, caffeine and pentoxifylline produced a 70% and 60% inhibition of ^{32}P incorporation, respectively.

3.2. *Study of the inhibition mechanism*

We studied the influence of two substrate concentrations on methylxanthine inhibition. Interaction between the substrates ATP and caffeine or pentoxifylline was investigated by determining the reaction rate at 4 different methylxanthine concentrations in the presence of 2 mg/ml casein and 4 different ATP concentrations from 2.5–10 μM. The Lineweaver Burk plot (fig.2) revealed a constant V_{max} and variable K_m, indicating that caffeine and pentoxifylline were competitive inhibitors of ATP. The K_i of caffeine and pentoxifylline were 3.8 mM and 5 mM, respectively.

Interaction of methylxanthines with casein was studied in the same manner, but in presence of constant ATP concentration (50 μM) and 5 different casein levels from 0.05–0.2 mg/ml. At ATP saturating level, a weak inhibition was observed for caffeine 10 mM. The Lineweaver-Burk plot (fig.3) indicated that caffeine and pentoxifylline were non-competitive inhibitors with respect to casein.

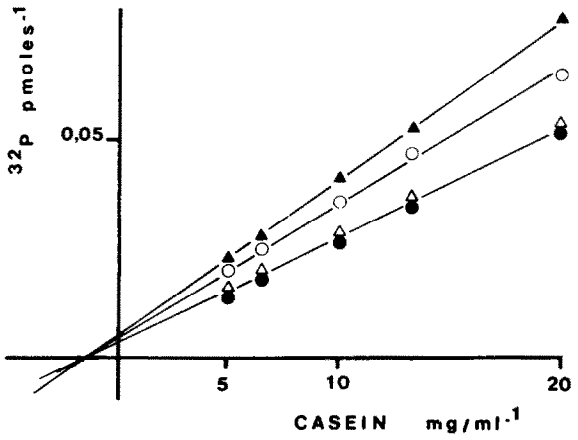

Fig.3. Lineweaver Burk plot of initial rate as function of casein concentrations: ATP, 50 μM; caffeine, (●——●) none; (△——△) 1 mM; (○——○) 5 mM; (▲——▲) 10 mM.

4. Discussion

These results clearly showed that the studied methylxanthine derivatives caffeine and pentoxifylline inhibit the human erythrocyte casein kinase activity. Analysis of inhibition mechanism revealed that caffeine and pentoxifylline were potent competitive inhibitors with respect to ATP. ATP saturating level decreased the inhibition rate. This suggested strongly that caffeine and pentoxifylline were bound to the ATP site on the enzyme. This would be expected since the methylxanthines are adenine analogues. Other kinases, such as human erythrocyte phosphatidyl inositol kinase [3] and thymidine kinase [4], are inhibited by caffeine.

Two types of protein kinase activities, cAMP-dependent and cAMP-independent can be distinguished. So far, only the methylxanthine action on cAMP-dependent phosphorylation is known. This action is generally attributed to the inhibition of phosphodiesterase activity. Here we clearly demonstrate that methylxanthines act as competitive inhibitors with respect to the substrate ATP site of a purified cAMP-independent protein kinase.

References

[1] Butcher, R. W. and Sutherland, E. W. (1962) J. Biol. Chem. 237, 1244–1250.
[2] Boivin, P. and Galand, C. (1979) Biochem. Biophys. Res. Commun. 89, 7–16.
[3] Buckley, I. T. (1977) Biochim. Biophys. Acta 498, 1–9.
[4] Sandlie, I. and Kleppe, K. (1980) FEBS Lett. 110, 223–226.