THE ENHANCEMENT OF POLYPEPTIDE SYNTHESIS IN MAMMALIAN SYSTEMS BY METHYLXANTHINES

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1. Introduction

A large number of methylxanthines including theophylline, aminophylline, caffeine, theobromine and some closely related compounds are important agents in medical use owing to their pharmacological properties: A number of cellular processes are enhanced by these compounds including the catecholamine effects on phosphorylase activation in liver [1] and muscle [2], and lipolysis in the adipose tissue [3]. Furthermore some methylxanthines cause a positive inotropism in the cardiac muscle [4], a relaxation in the smooth muscle [5] and a release of amylase in the parotid gland [6]. Effects of methylxanthines on cellular permeability, steroidogenesis [7] and enzyme synthesis [8] have also been described. The multiple effects of the methylxanthines have been extensively reviewed [9].

However, little is known about the biochemical effects of the methylxanthines at a molecular level, despite their extensive use in medicine. It has been shown that certain methylxanthines enhance the action of cyclic AMP (cAMP) by inhibiting the AMP phosphodiesterase activity [10]. The phosphodiesterase from different mammalian tissues in certainly affected by the methylxanthines but these compounds have no effect on the enzyme from unicellular organisms [11]. In any case, the observed inhibitory effect on phosphodiesterase does not explain some of the physiological effects of methylxanthines.

There are other reports showing that theophylline increases the release of nascent polypeptides from rat liver polysomes [12] and the amino acid incorporation into proteins by thyroid polysomes [13] but inhibits amino acid incorporation into proteins in the adrenal cortex [14]. Moreover, theophylline was without effect in a crude system from rat liver and brain for poly U-dependent polyphenylalanine synthesis, whereas aminophylline had a stimulatory effect in the same system [15].

To elucidate the effects that methylxanthines might have on protein synthesis we have tested the compounds in well-resolved cell-free systems for poly U-dependent phenylalanine incorporation. In this system there was no endogenous protein synthesis and the ribosomes were totally dependent upon the addition of a synthetic messenger and the elongation factors EF 1 and EF 2 for amino acid incorporation. The results presented in this contribution show that both aminophylline and theophylline strongly enhance polyphenylalanine synthesis. Such an effect was not observed with theobromine, but caffeine causes some stimulation. The stimulatory effect is dependent in all cases on the ribosome concentration, suggesting that the active methylxanthines act directly on the ribosomes, possibly causing some conformational change.

2. Materials and methods

Anaemia was induced in rabbits by using a 2.5% solution of phenylhydrazine, which was injected in different amounts on six sucessive days (1st day, 0.3 ml/kg; 2nd, 0.25 ml; 3rd 0.1 ml; 4th, 0.2 ml; 5th, 0.3 ml and 6th, 0.4 ml). The rabbits were bled on the seventh day under Nembutal anaesthesia and after heparin injection [16]. The blood was centrifuged for 5 min at 6000 rpm in a Sorvall GSA rotor to sediment the reticulocytes. These were then washed

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three times and lysis was carried out as described [16]. This lysate was centrifuged for 20 min at 30 000 *g* to remove white cells and membranes. The supernatant was then centrifuged for 2 hr at 180 000 *g* to sediment the polysomes. Washed ribosomes were obtained from this polysome fraction by centrifugation through a discontinuous sucrose gradient [17].

A crude preparation of elongation factors was obtained by 30–70% ammonium sulphate fractionation of the standard S-100 reticulocyte fraction. EF 1 activity was resolved from EF 2 by Sephadex G-200 column chromatography and purified by hydroxyapatite treatment [18]. The EF 2 activity recovered from the Sephadex G-200 chromatography was further purified by sequential processing in DEAE-cellulose chromatography, hydroxyapatite treatment [19] and phosphocellulose chromatography [20].

Yeast tRNA (Boehringer) was charged with [^{14}C]-phenylalanine (476 mCi/mmol New England Nuclear) in the presence of a crude supernatant fraction containing the synthetase activity. [^{14}C]Phe-tRNA was recovered by phenol treatment and ethanol precipitation, and further purified by BD-cellulose chromatography [21].

The assay for poly U-directed [^{14}C]phenylalanine incorporation was carried out in 0.1 ml mixtures containing 50 mM Tris–HCl buffer, at pH 7.4, containing 11 mM $MgCl_2$, 60 mM KCl, 8–10 mM 2-mercaptoethanol, 50 μg/ml poly U, 0.5 mM GTP, 1–2 pmoles reticulocyte ribosomes, 150 μg protein of the crude preparation of elongation factors and 6 pmoles of [^{14}C]Phe-tRNA. The incubation was carried out at 37°C for 15 min and ended by addition of 100 μg bovine serum albumin and 2.5 ml of cold 5% trichloroacetic acid. After heating for 10 min at 90°C, the mixtures were filtered through Whatman glass fiber filters (GF/A), the filters were dried under an infrared lamp, and radioactivity was estimated in a scintillation spectrometer.

3. Results

3.1. *Effect of aminophylline on polyphenylalanine synthesis at different concentrations of EF 2*

The stimulation of polyphenylalanine synthesis by aminophylline is clearly dependent on the ribosome concentration (table 1), since it was apparent only when ribosomes were the limiting component in the system. It certainly appears that aminophylline acts by stimulating the ribosome activity rather than by directly affecting the elongation factors, since it has no effect at a ribosome-saturating concentration when

Table 1
Effect of aminophylline on polyphenylalanine synthesis at different ribosome concentrations

Ribosomes (pmoles)	Aminophylline	pmoles [^{14}C]Phe-tRNA incorporated	% Control
0.28	–	0.617	
0.28	+	1.605	260
0.70	–	1.818	
0.70	+	3.424	188
1.40	–	3.185	
1.40	+	4.028	126
3.50	–	3.784	
3.50	+	4.042	107

Components and incubation conditions were as described in Materials and methods. Aminophylline concentration was 5 mM.

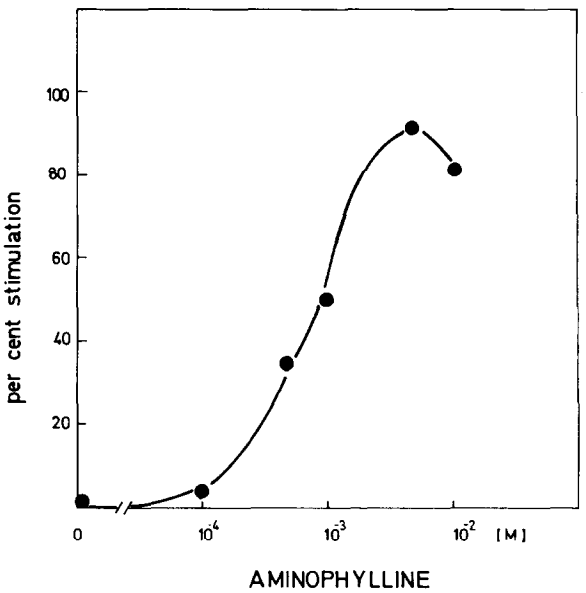

Fig. 1. Effects of different aminophylline concentrations on polyphenylalanine synthesis. Conditions as described in Materials and methods. 1.21 pmoles [^{14}C]phenylalanine were incorporated in the control.

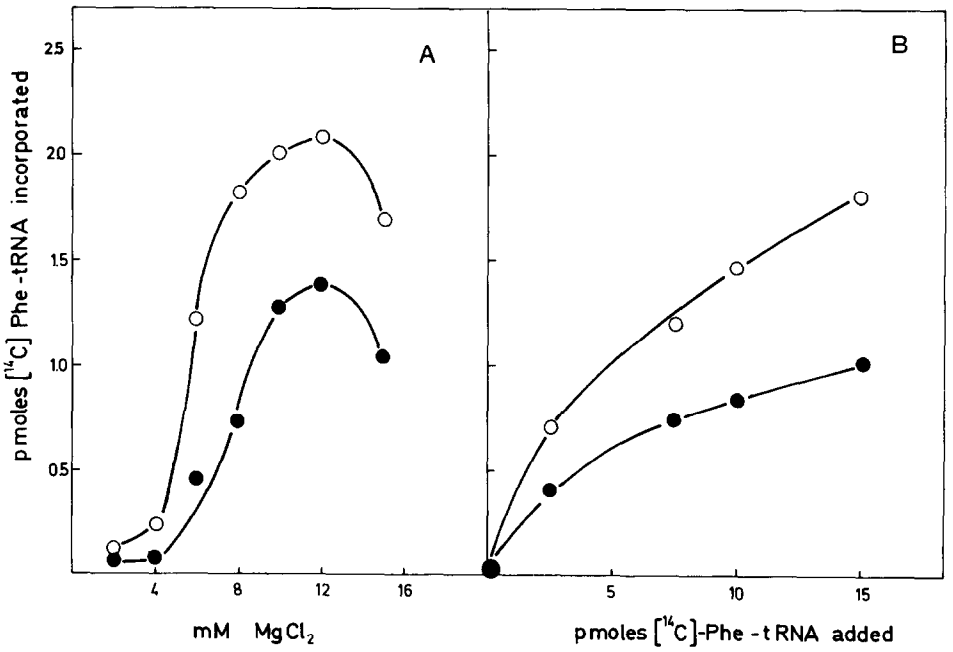

Fig. 2A. Dependence of polyphenylalanine synthesis upon magnesium chloride concentration. Fig. 2B. Dependence of aminophylline stimulation upon different [^{14}C]Phe-tRNA concentrations. Conditions in fig. 2A and B were as described in fig. 1. (●—●) Control. (○—○) Plus 5 mM aminophylline.

the limiting components are EF 1 and EF 2 (results not shown).

A certain effect was observed at aminophylline concentrations of 0.5 mM. The enhancement was increased with the concentration of aminophylline up to a maximal effect at 5 mM concentration (fig. 1). The enhancement by aminophylline is not due to a displacement in the optimal requirements of either magnesium or substrate concentration (fig. 2). Optimal polyphenylalanine synthesis was observed at 11–12 mM Mg^{2+} both in the presence and in the absence of aminophylline (fig. 2A). However, a stimulatory effect by aminophylline was observed at all magnesium concentrations studied. Similarly the enhancement by aminophylline was observed at all the concentrations of [^{14}C]Phe-tRNA tested (fig. 2B). Results not presented here also showed that the enhancement by aminophylline is not dependent on the GTP concentration.

3.2. *Comparative effects of methylxanthines*

The effect of a number of methylxanthines was tested in identical conditions on polyphenylalanine synthesis (table 2). The results obtained clearly show a strong enhancement by theophylline similar to that as aminophylline. A stimulatory effect was also observed to a lesser extent in the presence of caffeine, but no significant effect was found in the presence of theobromine.

Table 2
Effects of different methylxanthines on polyphenylalanine synthesis

System	pmoles	% Control
Complete	2.23	100
+ aminophylline	4.22	187
+ theophylline	4.02	178
+ caffeine	3.48	151
+ teobromine	2.56	113

Conditions as described in table 1. Methylxanthine concentration was 5 mM.

4. Discussion

Studies on the molecular action of methylxanthines have focussed their attention on their competitive inhibition of the cyclic AMP phosphodiesterase [9,10]. However, most of the pharmacological effects of methylxanthines cannot be explained only on the basis of their inhibitory effect on this enzyme [9]. It is therefore of interest to note a report showing that aminophylline stimulates protein synthesis by crude ribosomal systems from rat liver and brain, with the use of either poly U or endogeneous mRNA [15].

The results presented in this contribution show that aminophylline certainly causes a strong enhancement in poly U-directed phenylalanine incorporation in well-resolved cell-free systems from reticulocytes. Maximal stimulation by aminophylline in this system is observed when the ribosomes are the rate-limiting component in protein synthesis. Our data certainly suggest that aminophylline interacts with the ribosome, enhancing its activity in translation, even though aminophylline is not, under physiological conditions, a factor required for protein synthesis. It is indeed interesting that such a small molecule as aminophylline should have this positive effect on the ribosome. We are extending our work in order to elucidate the step(s) in translation specifically enhanced by aminophylline.

Theophylline has been shown to be a more efficient inhibitor of cyclic AMP phosphodiesterase than caffeine or theobromine [10]. Similarly, in our system, aminophylline and theophylline have the strongest stimulating action on polyphenylalanine synthesis, the effect being smaller in the case of caffeine and practically nil when theobromine is used. However, the positive effect of methylxanthines on polyphenylalanine synthesis in our system is not due to an increase in cyclic AMP, through their inhibitory effect on phosphodiesterase, since cyclic AMP was without effect, in our resolved system for polyphenylalanine synthesis (results not shown).

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