Divergent Effects of Theophylline on Adenylate Cyclase Preparations from Guinea-Pig Heart and Lung

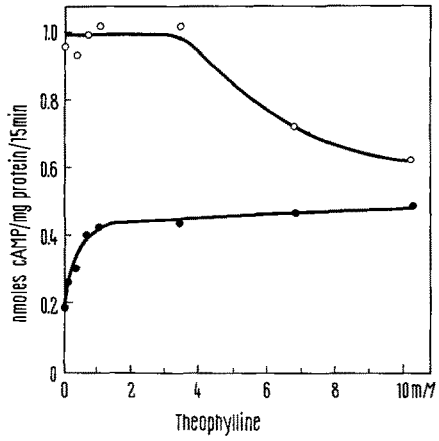
It has recently been demonstrated that high concentrations ($\geqslant 20$ mM) of theophylline, an inhibitor of adenosine 3' 5' monophosphate (cyclic AMP) phosphodiesterase, inhibit the basal and epinephrine-stimulated adenylate cyclase activities of rat erythrocyte ghosts[1]. In the course of investigations concerning the tissue specificity of adenylate cyclases, we noted that concentrations as low as 6–10 mM theophylline inhibited adenylate cyclase from particulate fractions of guinea-pig lung, but did not affect cyclase activity from guinea pig heart. The details and implications of this study are presented below.

Ventricular muscle and lung alveolar tissue from normal guinea-pigs were used. Tissues were minced, homogenized with 4 ml of chilled buffer containing 1 mM $MgCl_2$ (Mallinckrodt) and 2mM glycylglycine (Schwartz/Mann), pH 7.5, per g wet wt., and centrifuged at 1000 g for 15 min. The pellets were washed once with glycylglycine-$MgCl_2$ buffer, resuspended in buffer, and stored in small aliquots under liquid nitrogen until assayed for cyclase activity. Assay mixtures contained 1.2 mM ATP (Calbiochem, $3–5 \times 10^6$ cpm [α-^{32}P] ATP (International Chemical and Nuclear Corp.), 1.8 mM $MgCl_2$, 0.8 mM glycylglycine, 32 mM *Tris* (pH 7.8) (Sigma), theophylline (Schwartz/Mann) and/or unlabeled cyclic AMP (Schwartz/Mann), if desired, and approximately 150 µg (lung) or 300 µg (heart) enzyme fraction protein, in a total volume of 0.59 ml. After incubation for 15 min at 37°C the reaction was stopped and the [^{32}P] cyclic AMP in the assay mixture was isolated, with minor modifications, by the procedure of KRISHNA, WEISS and BRODIE[2], as described by LEVEY and EPSTEIN[3]. Heart and lung cyclase activities (nmoles [^{32}P] cyclic AMP measured per mg protein per 15 min) were increased above basal values 5- to 8-fold and 3- to 4-fold, respectively, by 9 mM fluoride (Matheson, Coleman and Bell). Isoproterenol (Schwartz/Mann) stimulated the heart enzyme activity up to 80% at 10^{-6}-$10^{-4}M$, and that of lung enzyme about 60%.

The Figure shows typical responses of heart and lung adenylate cyclase preparations to concentrations of theophylline up to approximately 10 mM. All theophylline concentrations tested increased apparent heart cyclase activity ([^{32}P] cyclic AMP content per mg protein per 15 min incubation, in the presence of phosphodiesterase activity) whereas that of lung cyclase was inhibited by 6–10 mM theophylline and unaffected by lower concentrations. A possible interpretation of these data assigns the apparent increase in heart cyclase activity to the inhibition by theophylline of residual cyclic AMP phosphodiesterase; the lung preparation may contain negligible phosphodiesterase and thus no theophylline effect was seen until concentrations had become high enough to inhibit the cyclase. Determinations of the phosphodiesterase activity of the cyclase preparations by the method of BROOKER et al.[4], using physiological concentrations of cyclic AMP ($1.6 \times 10^{-7}M$), revealed, as expected, much higher activities in heart (0.28 nmoles/mg protein/15 min) than in lung (0.07 nmoles/mg/15 min) fractions[5].

Unlabeled cyclic AMP (1mM)[1,6] was as effective as 10 mM theophylline in protecting nascent labeled cyclic AMP from phosphodiesterase action in the heart cyclase assays. The use of cyclic AMP plus theophylline gave the same activity as observed with either alone, indicating the absence of any inhibitory or stimulatory effect of 10 mM theophylline on heart cyclase, and that the increase in cyclic AMP in the presence of 0.1–10 mM theophylline was attributable solely to the inhibition of phosphodiesterase activity. The presence of 1 mM cyclic AMP in the lung cyclase assays did not significantly affect the activity (< 5% change), nor did cyclic AMP alter the inhibition observed in the presence of theophylline. These results are compatible with the very low phosphodiesterase activity of the lung cyclase fractions and with the inhibition by theophylline of lung cyclase discussed above.

The differential inhibition by theophylline of adenylate cyclases from lung and heart tissues demonstrated above suggests that subtle differences may exist in the catalytic moiety of adenylate cyclases from different tissues. This possibility has received little if any attention in the literature. As a practical matter, since this investigation has shown concentrations of theophylline of 10m M or less may inhibit cyclases in some tissues, the routine use of ca. 10 mM theophylline in assay procedures for adenylate cyclases may result in decreased activity in some cases. The effect of theophylline should be determined explicitly for the particular cyclase preparation of interest.



Response of adenylate cyclase preparations from guinea-pig heart and lung to theophylline. Points represent duplicate determinations; range, ±10%. ○, lung cyclase; ●, heart cyclase.

The essentially phosphodiesterase-free lung cyclase fractions would seem to be especially useful in studies of effects upon adenylate cyclase of phosphodiesterase-active compounds.

Zusammenfassung. Theophyllin verringert die Aktivität der Adenyl-Cyclase (AC) in der plasmamembranreichen Fraktion der Meerschweinchenlunge in Konzentrationen von 6–10 mM; es hat dagegen keinen Einfluss auf die Aktivität der AC in Meerschweinchenherzen. Diese Resultate weisen darauf hin, dass in verschiedenen Geweben geringe Unterschiede in der Enzym-Katalyse bestehen können.

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