PARADOXICAL STIMULATION BY 1-METHYL-3-ISOBUTYLXANTHINE OF RAT LIVER CYCLIC AMP PHOSPHODIESTERASE ACTIVITY

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1. Introduction

Xanthines are well-known inhibitors of cyclic nucleotide phosphodiesterases and among them, 1-methyl-3-isobutylxanthine (MIX) is the most potent [1]. In detailed kinetic studies, MIX has been shown to inhibit competitively the calmodulin-activated phosphodiesterase from porcine coronary arteries [2]. MIX is now very commonly used in intact cells to inhibit the degradation of cyclic nucleotides and raise their concentrations [3]. Here, we demonstrate a distinct, direct mechanism of activation by MIX of an isolated positively cooperative form of the soluble rat liver phosphodiesterases, the cGMP-stimulated enzyme [4,5]. The latter mechanism is quite different from the one characterizing cGMP activation [6].

2. Materials and methods

The rat liver cGMP-stimulated phosphodiesterase was prepared as in [6], except that all preparative buffers contained the following protease inhibitors: phenylmethylsulfonyl fluoride, 75 mg/l (solubilized first in 1.5 ml dimethylsulfoxide), benzamidine, 0.1 mM, leupeptin, 5 μM. Phosphodiesterase activity was assayed as in [6]. 5′-Nucleotidase (*Crotalus atrox* venom), phenylmethylsulfonyl fluoride, benzamidine and 1-methyl-3-isobutylxanthine were from Sigma (St Louis MO). Leupeptin was supplied by Peptide Institute (Osaka). 6-Chloropurineriboside 3′,5′-monophosphate was a generous gift of Dr Jastorff (University of Bremen).

Abbreviations: MIX, 1-methyl-3-isobutylxanthine; cAMP, adenosine 3′,5′-monophosphate; cGMP, guanosine 3′,5′-monophosphate

3. Results

Analysis of the cGMP-stimulated phosphodiesterase reveals a positively cooperative kinetic behavior with respect to cAMP as substrate. At 3 μM cGMP shifts the cooperative kinetic behavior of the enzyme to a normal Michaelis-Menten system and increases the affinity of the phosphodiesterase for cAMP [4,5,7]. At low concentrations (3–50 μM), MIX stimulated basal cAMP phosphodiesterase activity (fig.1). This particular effect of the xanthine was only detected in the absence of cGMP and at low cAMP substrate levels (3–5 μM). The maximal stimulation (200–300% of control activity depending on the preparation) was obtained at ~50 μM MIX (fig.1). At higher [cAMP] (100 μM), 50 μM MIX decreased cAMP hydrolysis (table 1). When cGMP was added to the incubation medium, the stimulatory effect of MIX was markedly reduced: in the presence of 0.2 μM cGMP, the stimulatory effect of MIX was barely detectable, and the inhibitory effect of 0.1 or 0.3 mM MIX was strengthened (table 2). Consequently, the factor of cGMP stimulation was progressively reduced as the concentration of the xanthine increased: at 0.3 mM MIX, the cGMP-activating effect (at 0.2 μM) was no longer observed (table 2). In the presence of 3 μM cGMP, the cAMP phosphodiesterase activity exhibited a linear double-reciprocal plot and MIX (over 0.01–0.2 mM) showed linear competitive inhibition with respect to cAMP (K_i = 18 μM, fig.2). A similar mechanism of inhibition and equipotent K_i-values were obtained with cGMP as substrate over 5–100 μM (not shown).

The two effects of MIX (stimulation and inhibition) were reproduced with 50 μM 6-chloropurineriboside 3′,5′-monophosphate, an analogue of the substrate and potent inhibitor [6], but not with

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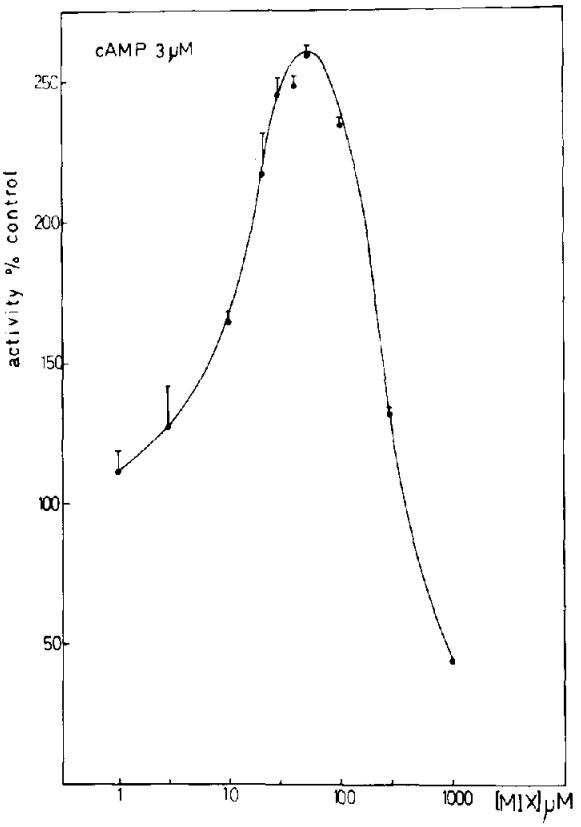

Fig. 1. Dose–response curve between the stimulation of cAMP phosphodiesterase activity and the concentration of 1-methyl-3-isobutylxanthine (MIX). cAMP was 3 μM. Results are expressed as % of the control ± SD.

0.2 μM cGMP for which only the stimulatory effect was observed (table 1). Complete substrate–velocity relationships indicated that MIX, 6-chloropurine-riboside 3′,5′-monophosphate and cGMP all attenuated

Table 2
Effect of MIX on the activity of the cGMP-stimulated phosphodiesterase

Addition	Phosphodiesterase act. (pmol . min^{-1}. mg protein^{-1})	
	Control	cGMP (0.2 μM)
None	149.7 ± 6.2	754.0 ± 7.7 (5.0)
MIX		
0.01 mM	231.3 ± 5.0	872.1 ± 21.1 (3.8)
0.03 mM	301.6 ± 12.1	695.9 ± 13.8 (2.3)
0.1 mM	250.4 ± 11.2	327.3 ± 12.4 (1.3)
0.3 mM	110.7 ± 9.5	118.5 ± 1.8 (1.1)

cAMP phosphodiesterase activity was assayed at 3 μM cAMP as substrate, in the absence and presence of 0.2 μM cGMP (effector). Results are means of triplicates ± SD. The factor of activation by cGMP is given in parenthesis

the positively cooperative character of the enzyme. However, in contrast to cGMP, the xanthine or the cAMP analogue did not decrease the app. K_m-value for cAMP.

4. Discussion

The stimulatory effect of MIX on cAMP hydrolysis by the cGMP-stimulated phosphodiesterase could appear as a paradox, since xanthines are well-known as inhibitors of phosphodiesterases of mammalian origin [1]. Actually, this observation is not unexpected: theoretical enzymology shows that a competitive inhibitor can increase the velocity of a positively cooperative enzyme provided the competitor mimics the cooperative substrate binding and that low con-

Table 1
Effect of MIX, 6-chloropurineriboside 3′,5′-monophosphate and cGMP on cAMP phosphodiesterase activity (pmol cAMP hydrolyzed . min^{-1}. mg protein^{-1})

Substrate cAMP (μM)	Control	MIX (50 μM)	6-Chloropurine-riboside 3′,5′-monophosphate (50 μM)	cGMP (0.2 μM)
5	152 ± 39	314 ± 7	609 ± 13	696 ± 29
10	531 ± 5	772 ± 15	1192 ± 8	1621 ± 95
25	2784 ± 23	2072 ± 65	2649 ± 9	4337 ± 39
50	5829 ± 98	3658 ± 81	4336 ± 109	6886 ± 260
100	7650 ± 73	5401 ± 53	6069 ± 49	7983 ± 147

Results are means of triplicates ± SD

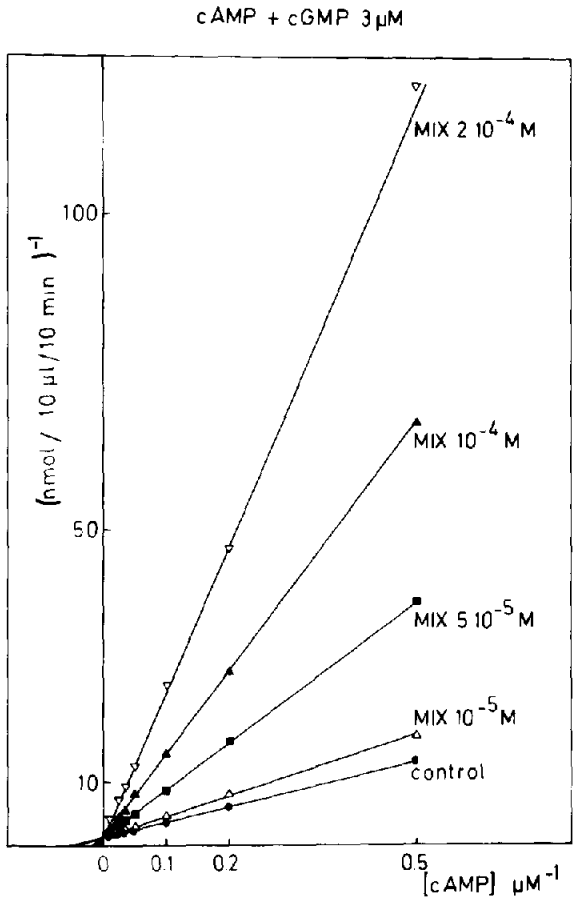

Fig.2. Inhibitory effect of 1-methyl-3-isobutylxanthine (MIX) on cAMP hydrolysis (double-reciprocal plot). Activity was measured in the presence of 3 μM cGMP (effector). Substrate ranges shown are 2–100 μM for cAMP. Results are means of triplicate determinations and are representative of experiments with 3 different preparations.

centrations of both the substrate and the competitor are used [8]. This phenomenon can occur in the case of concerted or sequential cooperativity [8,9] and has already been observed for other allosteric enzymes, e.g., aspartate transcarbamoylase (stimulated by substrate analogues) [10]. The stimulatory effect of MIX has properties consistent with the theoretical predictions: it is only observed at low concentrations of both the substrate cAMP and the xanthine and is associated with an attenuation of the cooperative interactions inside the enzyme. Furthermore, it is no longer detected when the positive cooperativity of the enzyme is partially or completely abolished, that is when cGMP is the substrate or when cGMP activates cAMP hydrolysis. In these last conditions MIX is a pure inhibitor: double-reciprocal plots demonstrate the competitive nature of this inhibition (fig.2).

As shown in table 1, only MIX, but not cGMP, has a marked inhibitory effect at high cAMP concentration (100 μM). Since the effect of the xanthine can be entirely explained by its binding to catalytic sites interacting with each other in a positively cooperative way, the effect of cGMP must follow another mechanism of activation presumably involving a distinct regulatory site [8]. Evidence for such a regulatory site was recently obtained with the use of cyclic nucleotide derivatives [6].

As shown in table 2, the inhibition of the phosphodiesterase by MIX is much more potent in the presence than in the absence of cGMP. This could be interpreted in two ways:

(i) cGMP bound to the regulatory site increases the affinity of catalytic sites for MIX; or
(ii) MIX, in addition to being a competitive inhibitor of the catalytic sites, is a competitive antagonist blocking the binding of cGMP to its activatory site.

Our kinetic data cannot distinguish between these two interpretations.

In conclusion, these results are consistent with a competitive interaction of MIX at the catalytic sites of the rat liver cGMP-stimulated phosphodiesterase. An additional competitive interaction with the cGMP regulatory site cannot be excluded by our data but is not necessary to explain them. The mechanism of MIX stimulation described here is quite different from the long-term mechanism of activation by MIX of a cAMP-specific phosphodiesterase in intact human lymphocytes [11]. Since it is observed at rather low concentrations, it is tempting to speculate that some of the pharmacological properties of the xanthines might be related to activatory rather than inhibitory effects on the phosphodiesterases [12].

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