XANTHINE OXIDASE – XANTHINES AS A SYSTEM OF ENDOGENOUS REGULATION OF PHOSPHODIESTERASE ACTIVITY

L. A. Kozhemyakin, T. R. Koroleva, and D. S. Korostovtsev

UDC 612.015.1.577.152.1].014.46: 547.857.4

KEY WORDS: xanthine oxidase; phosphodiesterase; cyclic nucleotides; rat liver.

Regulation of the content of cyclic nucleotides is an important problem in clinical and experimental biochemistry. One way in which the cyclase system can be influenced is through the use of methylxanthines, which are inhibitors of phosphodiesterase (PDE).

The data given below enable an approach to be made to the study of interaction between PDE and methylxanthine in connection with an examination of the role of the endogenous xanthine – xanthine oxidase (XOase) – in the regulation of PDE activity and, consequently, of the cyclic AMP/cyclic GMP content in the tissues in vivo.

EXPERIMENTAL METHOD

Activity of PDE and XOase and the cyclic AMP content were determined in albino rat liver tissue homogenates. PDE activity was determined by Poch's method [12], which is based on determination of the degree of enzymic hydrolysis of cyclic AMP-^{3}H, followed by separation of the nucleotides by thin-layer chromatography on Silufol UV-254 disks [1]. XOase activity was estimated colorimetrically [9] with Folin's reagent. The cyclic AMP content was determined with the aid of kits from the Radiochemical Centre, Amersham, England. The protein concentration in the incubation medium was determined by Lowry's method [10]. Solutions of theophylline and allopurinol [4-hydroxypyrazolo(3,4-d)-pyrimidine] were added to the incubation mixture in concentrations indicated in Fig. 1 and Table 1. For the experiments in vivo, noninbred male albino rats weighing 150-200 g were used. The test substances were administered once daily in the following doses: allopurinol – 50 mg/kg, by the intragastric route; hypoxanthine – 50 mg/kg, intraperitoneally; ammonium molybdate – 5 mg/kg, by the intragastric route).

EXPERIMENTAL RESULTS

The results of the study of the action of theophylline on activity of the enzymes XOase and PDE in parallel tests on rat liver homogenate are given in Fig. 1. It will be clear from Fig. 1A that a significant fall in XOase activity took place in lower concentrations of theophylline (10^{-5}M) than the decrease in PDE activity (10^{-4}M). The curves of similar character reflecting changes in enzyme activity with time in the presence of a constant concentration of theophylline (5×10^{-3}M) are shown in Fig. 1B. According to these data, XOase activity began to decline after only 5 min, whereas a significant decrease in PDE activity was observed after 20 min. Increasing concentrations of theophylline, it can be tentatively suggested, led to a decrease in XOase activity, which always preceded a fall in PDE activity. This earlier change in XOase activity could be explained on the grounds that changes in PDE activity are secondary relative to those in XOase activity. Accordingly, in the next part of the work the effects of known activators and inhibitors of XOase on the PDE–cyclic AMP system were studied in vitro and in vivo.

The results of the study of the action of allopurinol, an effective preparation in the treatment of gout and an inhibitor of XOase, are shown in Table 1. It will be clear from Table 1 that allopurinol, although without any direct inhibitory action on PDE, effectively inhibits the PDE activity of the homogenate in vivo and in vitro. It must be emphasized that if high concentrations of the inhibitor were used in vitro and if allopurinol in a

Department of Biochemistry, S. M. Kirov Military Medical Academy, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 90, No. 12, pp. 673-675, December, 1980. Original article submitted February 8, 1980.

0007-4888/80/9012-1665$07.50

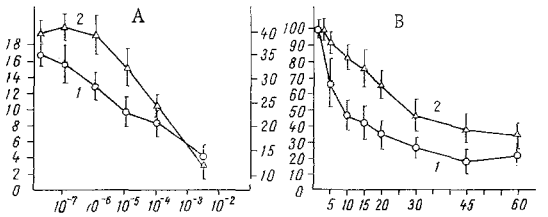

Fig. 1. Dynamics of changes in activity of XOase and PDE in rat liver tissue homogenate depending on theophylline concentration (A) and duration of incubation with 5×10^{-3} M theophylline (B). A: Abscissa, theophylline concentration in incubation medium (in M); ordinate: left – XOase activity (in moles xanthine/mg protein/15 min), on right–PDE activity (in pmoles cyclic AMP/mg protein/min). B: Abscissa, duration of incubation (in min); ordinate, enzyme activity (in % of initial level): 1) XOase, 2) PDE.

TABLE 1. Effect of Allopurinol in Experiments in vitro and in vivo on XOase and PDE Activity and on Cyclic AMP Concentration (M ± m)

Series of experiments	Experimental conditions	Number of animals	XOase activity, μmoles xanthine/mg protein/15 min	PDE activity, pmoles cyclic AMP/mg protein/min	Cyclic AMP concentration, nmoles/g tissue
In vitro	Administration of allopurinol				
	0	8	17,4±1,3	31,3±2,4	1,56±0,08
	5 μmoles	7	15,9±1,4	36,0±2,9	1,48±0,10
	50 μmoles	7	11,8±1,0*	19,4±2,0*	2,19±0,14*
	500 μmoles	9	4,1±0,5*	11,6±0,7*	3,21±0,26*
In vivo	Before administration	10	15,4±1,7	40,8±3,5	1,28±0,09
	1st Day of administration	10	16,8±1,6	34,5±2,6	1,16±0,07
	4 Days " "	11	10,2±0,7*	26,5±3,0*	1,87±0,19
	9 Days " "	11	6,9±0,8*	16,3±1,8*	2,66±0,30*

*Here and in Table 2, $P < 0.05$ compared with control.

TABLE 2. Effect of Adaptive Stimulation of XOase by Repeated Injections of Xanthine and Ammonium Molybdate on PDE Activity and Cyclic AMP Concentration in Rat Liver Tissue (M ± m)

Series of experiments	Experimental conditions	Number of animals	XOase activity, μmoles xanthine/mg protein/15 min	PDE activity, pmoles cyclic AMP/mg protein/min	Cyclic AMP concentration, nmoles/g tissue
Administration of xanthine	Before administration	10	14,6±1,6	29,0±3,1	1,16±0,21
	3 days of administration	8	16,7±1,4	33,2±3,6	1,22±0,15
	7 days " "	8	23,9±2,0*	40,0±3,8	0,82±0,27
	14 days " "	10	29,2±2,5*	51,6±4,3*	0,69±0,17*
Administration of ammonium molybdate	Before administration	9	21,3±1,8	38,1±3,1	1,08±0,11
	3 days of administration	10	22,0±1,9	35,6±2,4	1,22±0,25
	7 days " "	10	28,4±2,3*	44,3±3,0	1,05±0,16
	14 days " "	12	34,0±2,8*	64,2±5,3	0,54±0,08*

concentration of 50 mg/kg was given for a long time (9 days) in vivo, the cyclic AMP concentration rose significantly in the liver tissue.

Other workers [5, 7, 8] showed that XOase is an adaptive enzyme and its activity is significantly increased on the 10th-14th day of administration of the substrate for this enzyme – hypoxanthine or xanthine – to the animals. Table 2 gives the results of a parallel investigation of XOase and PDE activity during prolonged administration of xanthine to the animals, evidence that changes in XOase activity also precede changes in PDE activity.

It has been shown [6, 11] that Mo^{++} is a component of the active center of XOase. There is evidence that the enzyme is activated by molybdenum salts [2-4]. In the present experiments administration of ammonium molybdate to the animals was followed by significant activation of XOase, accompanied by a parallel and significant increase in PDE activity (Table 2).

The data described above thus show that there is close interaction between enzymes of the XOase and PDE systems. It can be tentatively suggested that PDE is regulated by the dynamic concentration of endogenous xanthines which, in turn, is determined by the intensity of reactions of the terminal stages of purine metabolism. Pharmacological action on PDE through the XOase–xanthines system may be an important way of modulating the concentrations of cyclic AMP and cyclic GMP in the tissues in clinical and experimental medicine.

LITERATURE CITED

1. D. R. Beritashvili and K. A. Kafiani, Vopr. Med. Khimii, No. 3, 322 (1975).
2. A. I. Kazachenko, L. S. Vartanyan, and É. M. Gonikberg, Biokhimiya, 36, 27 (1971).
3. V. V. Koval'skii and I. E. Vorotnitskaya, Dokl. Akad. Nauk SSSR, 187, 1422 (1969).
4. S. E. Severin, P. P. Filippov, and G. A. Kochetov, Usp. Sovrem. Biol., 69, 423 (1970).
5. L. A. Tiunov, N. M. Petushkov, and T. R. Koroleva, Byull. Éksp. Biol. Med., No. 5, 34 (1974).
6. E. Di Renzo, E. Kaleita, J. Oleson, et al., J. Am. Chem. Soc., 75, 753 (1953).
7. L. Dietrich, J. Biol. Chem., 211, 79 (1954).
8. P. Feigelson, M. Feigelson, and T. Wood, Science, 120, 502 (1954).
9. G. Litwack, J. Bottwell, J. Williams, et al., J. Biol. Chem., 200, 303 (1953).
10. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
11. B. Mackler, H. Mahler, and D. Green, J. Biol. Chem., 210, 149 (1954).
12. G. Poch, Arch. Exp. Pathol. Pharmak., 268, 272 (1971).

TRANSLATION OF MOUSE INTERFERON mRNA IN A CELL-FREE SYSTEM

U. Kiessling, T. M. Sokolova, and F. I. Ershov

UDC 612.429.015.3:547.963.32:576.858.095.383

KEY WORDS: interferon; synthesis; mRNA.

Interferon is an inducible protein with high antiviral activity (> 10^9 inhibitory units/mg protein). Progress in the study of the mechanisms of synthesis and action of interferon is largely bound up with the development of methods of testing its messenger RNA (mRNA). At the present time both whole cells and cell-free systems of protein synthesis are used for translation of interferon mRNA [2]. The latter open up new opportunities for the study of fine mechanisms of regulation of synthesis and action of interferon at the translation level.

Of the known eukaryotic translation systems the most effective known is a cell-free system consisting of rabbit reticulocyte lysate, in which low concentrations of mRNA (1-5 μg/ml) can be translated in the presence of ribosomal RNA, so that total preparations can be used. Treatment with micrococcal nuclease destroys endogenous templates and enables a system dependent upon added exogenous mRNA to be obtained [5].

This paper gives the results of a parallel study of translation of mouse interferon mRNA in a cell-free system from rabbit reticulocyte lysate and in tissue culture cells.

D. I. Ivanovskii Institute of Virology, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR V. M. Zhdanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 90, No. 12, pp. 675-677, December, 1980. Original article submitted June 6, 1979.