EFFECT OF PHENOTHIAZINES ON THE ACTIVITY OF GLYCOGEN PHOSPHORYLASE *b*

T. B. KTENAS, T. G. SOTIROUDIS, N. G. OIKONOMAKOS and A. E. EVANGELOPOULOS
The National Hellenic Research Foundation, 48 Vassileos Constantinou Avenue, Athens 501/1, Greece

Received 13 February 1978

1. Introduction

Chloroprothixene, a thioxanthene derivative, significantly prolongs ethanol action [1]. Strong inhibitory activity of a series of phenothiazines on Na, K-ATPase and horse liver alcohol dehydrogenase has been reported [2,3]. Chloropromazine and imipramine were also found to activate swine renal 15-hydroxyprostaglandin dehydrogenase [4].

Glycogen phosphorylase *b* (EC 2.4.1.1) shows an absolute requirement for AMP for enzymatic action [5]. It has been shown that AMP activation is enhanced by a variety of factors, e.g., substrate and buffer ions, anions, divalent metal ions and polyvalent organic cations [6–11].

In the present paper, we have studied the effect of a series of phenothiazine derivatives (all being pharmacologically active) upon the catalytic and structural properties of phosphorylase *b*. Our results indicate that some phenothiazines cause a significant enhancement of the binding of AMP to the enzyme.

2. Materials and methods

Phosphorylase *b* was isolated from rabbit skeletal muscles as in [12]. 2-Mercaptoethanol was used instead of L-cysteine in all steps of the isolation procedure. The enzyme was recrystallized four times and AMP was removed by passage through a column of Sephadex G-25. The enzyme concentration was measured spectroscopically using an extinction coefficient ($E^{1\%}_{1cm}$) at 280 nm of 13.2 [13]. Activity assays of phosphorylase *b* were carried out in the direction of glycogen synthesis [14]. Inorganic phosphate released in the reaction was measured as in [15]. No interference of phenothiazines, when tested at various concentrations, during colorimetric determination of phosphate was found [16]. Ultracentrifugation studies were performed on a MSE Centriscan 75, at a rotor speed of 60 000 rev/min and temp. 20°C. The enzyme concentration in all runs was 10 mg/ml.

In all experiments, a 4×10^{-2} M sodium β-glycerophosphate, 3×10^{-2} M 2-mercaptoethanol and 1×10^{-3} M EDTA buffer (pH 6.8) was used.

Promazine·HCl and chloropromazine·HCl were obtained from two sources: Smith, Kline and French Laboratories (Philadelphia, PA) and MS Chemicals (Milano, Italy). These two compounds were pure by thin-layer chromatography (ammonium hydroxide/benzene/dioxan, 5:60:35) [17]. Oyster glycogen, glycerophosphate and EDTA were purchased from BDH. Glucose-1-phosphate and AMP were products of Sigma. All other compounds and reagents were of the highest commercially available purity.

3. Results

The effect of increasing concentrations of promazine on the activity of phosphorylase *b* at various AMP concentrations is shown in fig.1. It can be seen that promazine stimulates AMP activation at various concentrations of the enzyme activator. Maximal activation at all AMP concentrations tested is attained at 4×10^{-3} M promazine. Promazine is most effective at lower AMP concentrations. After increasing promazine concentration above 4×10^{-3} M, stimulation of phosphorylase *b* activation by AMP is decreased. In all cases, however, maximum velocity of the enzyme is not affected by promazine.

The sigmoid response of the enzyme to AMP is

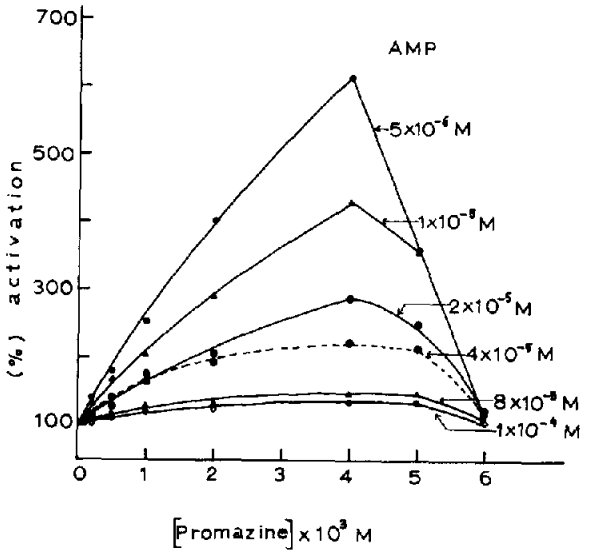


Fig.1. Effect of promazine on the activity of phosphorylase *b*. The enzyme (10 μg/ml) was assayed at 30°C with 1.6×10^{-2} M glucose-1-phosphate, 1% glycogen and various concentrations of AMP and promazine mentioned in the figure. The enzyme activities without promazine were taken as 100%.

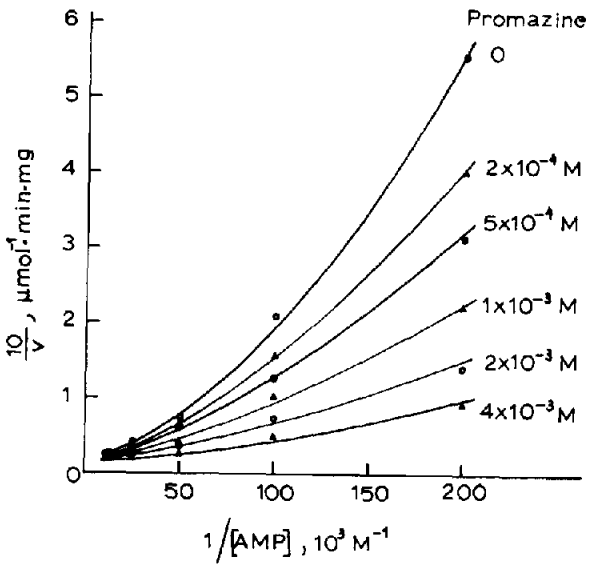


Fig.2. Lineweaver-Burk plots for AMP activation of phosphorylase *b*, in the presence of 2, 5, 10, 20 and 40×10^{-4} M promazine. Activities were expressed as μmol phosphate released/min/mg enzyme. The enzyme was assayed as in fig.1.

partially affected by increasing of promazine concentration in the assay system, as shown in the Lineweaver-Burk plots for AMP binding to phosphorylase *b* of fig.2. The Hill coefficient (n) and the K_m value for this nucleotide are also dependent on the promazine concentration (table 1). In contrast to its effect on K_m value for AMP, promazine has no effect on the affinity of the enzyme for the substrate, glucose-1-phosphate. At 1×10^{-3} M AMP and 1% glycogen, the presence of 4×10^{-3} M promazine does not affect the K_m value for glucose-1-phosphate (7.9×10^{-3} M).

Ultracentrifugation studies were performed to determine whether the dimer–tetramer equilibrium in phosphorylase *b* is affected by promazine. The following results were obtained:

(i) The enzyme in the presence of 5×10^{-4} M AMP has a sedimentation coefficient of 9.5 S, which corresponds mainly to the dimeric species [18].

(ii) In the presence of 2×10^{-3} M promazine and 5×10^{-4} M AMP the sedimentation pattern shows two peaks, one of 10.4 S (11.5%) and another of 12.4 S (88.5%) which correspond to an almost complete conversion of the enzyme to the tetrameric form. In contrast, promazine alone causes no alteration in the ultracentrifugal profile of phosphorylase *b*. A sedimentation coefficient of 8.4 S was found in the presence or absence of promazine (2×10^{-3} M) in agreement with [19].

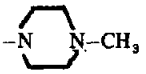
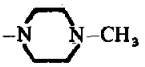
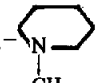
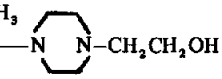
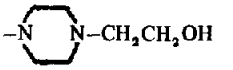
In order to detect structural requirements for the

Table 1
Effect of promazine on Hill coefficient (n) and K_m value for AMP binding to phosphorylase *b*

Ipromazine I ($M \times 10^3$)	K_m ($M \times 10^5$)	n
0	7.2	1.50
2	6.3	1.40
5	5.6	1.35
10	4.5	1.40
20	3.7	1.20
40	2.6	1.15
50	2.8	1.35
60	6.5	1.35

Affinity and interaction parameters were calculated as in [20]

Table 2
Effect of structural analogues of promazine to phosphorylase *b* activity

Compound	Structure	% Activation
		
Promazine	R_1=–H; R_2=–CH_2CH_2–N(CH_3)$_2$	204
Chloropromazine	R_1=–Cl; R_2=–CH_2CH_2–N(CH_3)$_2$	191
Promethazine	R_1=–H; R_2=–$CH(CH_3)$–N(CH_3)$_2$	76
Perazine	R_1=–H; R_2=–CH_2CH_2– 	106
Trifluoperazine	R_1=–CF_3; R_2=–CH_2CH_2– 	94
Thioridazine	R_1=–SCH_3; R_2=–CH_2– 	102
Perphenazine	R_1=–Cl, R_2=–CH_2CH_2– 	100
Fluphenazine	R_1=–CF_3; R_2=–CH_2CH_2– 	105

The enzyme (10 μg/ml) was assayed with 1.6×10^{-2} M glucose-1-phosphate, 1% glycogen, 2×10^{-5} M AMP and 2×10^{-3} M of each analogue. The enzyme activity without the analogue was taken as 100%

mechanism of promazine action, a series of promazine analogues were tested in experiments similar to those utilized for demonstrating stimulation of AMP activation by promazine (table 2).

4. Discussion

Promazine enhances the binding of AMP to glycogen phosphorylase *b* as judged by the kinetic experiments and the formation of the tetrameric species jointly with the nucleotide (fig.1, table 1). This stimulation of AMP binding to the enzyme is possibly due to an interaction of the negatively-charged groups of the protein molecule with the cationic group of the side-chain of the drug. In fact, such electrostatic interactions between phenothiazines and bovine serum albumin or bile salts have been reported [21,22]. As can be seen from the effects of various analogues of promazine upon the activity of phosphorylase *b* (table 2), the nature of the side-chain plays an important role for stimulation of enzyme activity. Besides the side-chain contribution, the observed phosphorylase *b* activation could have arisen also from a hydrophobic interaction between the apolar three ring system of the promazine molecule with a hydrophobic area of the enzyme molecule.

Acknowledgement

Supported by the National Hellenic Research Foundation.

References

[1] Scheckel, C. L. (1969) in: The Thioxanthenes. Mod. Probl. Pharmacopsychiat. (Lehman, H. E. and Ban, T. A. eds) vol. 2, pp. 1–14, Karger, Basel, New York.
[2] Brody, T. M., Akera, T., Baskin, S. I., Gubitz, R. and Lee, C. Y. (1974) Ann. NY Acad. Sci. 242, 527–542.
[3] Skurský, L., Kovár, J. and Michalský, J. (1975) FEBS Lett. 51, 297–299.
[4] Tai, H.-H. and Hollander, C. Y. (1976) Biochem. Biophys. Res. Commun. 68, 814–820.
[5] Green, A. A. and Cori, G. T. (1943) J. Biol. Chem. 151, 21–29.
[6] Kastenschmidt, L. L., Kastenschmidt, J. and Helmreich, E. (1968) Biochemistry 7, 4543–4556.
[7] Sealock, R. W. and Graves, D. J. (1967) Biochemistry 6, 201–206.
[8] Madsen, N. B. (1965) in: Muscle Symposium (Paul, W. M., Daniel, E. E., Kay, C. M. and Monckton, G. eds) p. 112, Pergamon, Oxford.
[9] Krebs, E. G. (1954) Biochim. Biophys. Acta 15, 508–515.
[10] Wang, J. H., Humniski, P. M. and Black, W. J. (1968) Biochemistry 7, 2037–2044.
[11] Mott, D. M. and Bieber, A. L. (1970) J. Biol. Chem. 245, 4058–4066.
[12] Fischer, E. H. and Krebs, E. G. (1962) Methods Enzymol. 5, 369–373.
[13] Buc, M. H., Ullmann, A., Goldberg, M. and Buc, H. (1971) Biochimie 53, 283–373.
[14] Illingworth, B. and Cori, G. T. (1953) Biochem. Prep. 3, 1–9.
[15] Fiske, C. H. and SubbaRow, Y. (1925) J. Biol. Chem. 66, 375–400.
[16] El-Dorry, H. F. A., Medina, H. and Bacila, M. (1972) Anal. Biochem. 47, 329–336.
[17] Clarke, E. G. C. (1969) in: Isolation and Identification of Drugs (Clarke, E. G. C. ed) pp. 517–518, The Pharmaceutical Press, London.
[18] Black, W. J. and Wang, J. H. (1968) J. Biol. Chem. 243, 5892–5898.
[19] Assaf, S. A. and Yunis, A. A. (1971) Biochem. Biophys. Res. Commun. 42, 865–870.
[20] Madsen, N. B. and Schechosky, S. (1967) J. Biol. Chem. 242, 3301–3307.
[21] Keeler, J. D. and Sharma, R. P. (1974) Biochem. Pharmacol. 23, 2679–2687.
[22] Carey, M. C., Hirom, P. C. and Small, D. M. (1976) Biochem. J. 153, 519–531.