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THE ACTION OF CHLORPROMAZINE ON YEAST HEXOKINASE

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Abstract—The effect of chlorpromazine on yeast hexokinase was investigated in a reaction system in which the activity of the kinase enzyme, coupled to glucose-6-phosphate dehydrogenase, was determined by measuring spectrophotometrically the reduction of TPN. Hexokinase activity, either in the presence or absence of chlorpromazine, showed great sensitivity to the magnesium–ATP ratio.

Concentrations of chlorpromazine from $8{\cdot}5 \times 10^{-6}$M to $6{\cdot}8 \times 10^{-5}$M either greatly activated or inhibited hexokinase activity, depending on the level of magnesium–ATP in the system.

Implications of the observed effects of CPZ on hexokinase are discussed in relation to some of the pharmacological activities of the drug.

INTRODUCTION

HYPOTHERMIA and hyperglycemia have been reported in laboratory animals[1–3] and human subjects[4–5] receiving chlorpromazine (CPZ).* Many studies have shown that CPZ inhibits oxygen uptake in various tissues,[6–7] but little evidence of a direct effect of CPZ on the substrate phosphorylation reactions involved in glucose utilization has been discerned. Various workers have shown that in mitochondrial preparations CPZ uncouples oxidative phosphorylation[8–11] and inhibits the activity of ATPase,[8,11,12] cytochrome oxidase,[8,12] and reactions coupled to the oxidation of DPNH.[13] Thus, it would seem possible that any effect of CPZ on anaerobic carbohydrate metabolism might be through action of the drug on enzyme systems associated with phosphorylation reactions.

Earlier work in this laboratory showed that administration of CPZ *in vivo* decreased the formation of pyridoxal-5-phosphate from ATP and pyridoxal in brain homogenates from normal and vitamin B_6-deficient rats.[14] These results were interpreted as an indication that CPZ prevented the transfer of phosphate from ATP to pyridoxal by inhibiting the action of the enzyme pyridoxal kinase. Since the pyridoxal kinase system resembles the phosphokinase reactions occurring in anaerobic glycolysis, studies were undertaken to test the effect of CPZ on a glycolytic kinase.

Although the work previously done with CPZ has principally involved various mammalian cell preparations and enzymes, some work has been reported in which glycolytic enzymes from yeast were used.[9] These preparations are readily available in purified form and offer the further advantage of being well characterized. For these

* The following abbreviations are used: chlorpromazine-HCl (10-[3-dimethylaminopropyl]-2-chlorophenothiazine-HCl) (SKF no. 2601-A), CPZ; adenosine-5′-triphosphate, ATP; diphosphopyridinenucleotide, oxidized and reduced form, DPN and DPNH, respectively; triphosphopyridine nucleotide, oxidized and reduced form, TPN and TPNH, respectively; dinitrophenol, DNP.

reasons the present study was carried out using yeast hexokinase rather than a mammalian preparation.

EXPERIMENTAL METHODS

Determination of enzyme activity

The activity of hexokinase was measured by coupling the kinase reaction to glucose-6-phosphate dehydrogenase (*Zwischenferment*) and determining spectrophotometrically the change in absorbency at 340 mμ due to the reduction of TPN:

glucose + ATP → glucose-6-phosphate + TPN → 6-phosphoglucono-δ-lactone + TPNH

The velocity of the initial phase of the reaction (during the first 60 or 90 sec) was used as an index of hexokinase activity. In this system the concentration of the *Zwischenferment* was maintained in excess over the hexokinase concentration, thereby insuring that the rate of the phosphorylation reaction limited the rate of TPN reduction (Fig. 1).

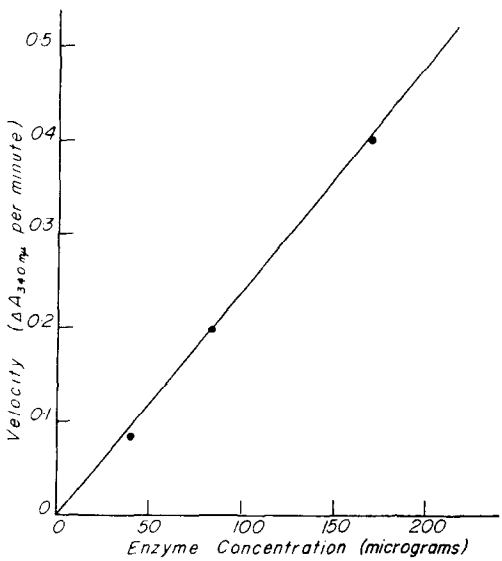


FIG. 1. Effect of enzyme concentration on the rate of TPN reduction.

All reagents were obtained commercially.* The reaction system contained: tris-(hydroxymethyl)aminomethane, 0·05 M, pH 7·5; potassium dihydrogenphosphate 0·005 M; crystalline yeast hexokinase, 0·04 mg; *Zwischenferment*, 0·75 mg; glucose, 0·1 M; TPN, 2×10^{-4}M; ATP and magnesium chloride. The final reaction volume in the cuvettes was 3·0 ml. In order to determine the effect of CPZ, various concentrations of the drug were added to the enzyme solution. After incubation for 15–20 min at room temperature, 0·2 ml of the enzyme–CPZ solutions were added to the reaction system containing all components except ATP. The reaction was initiated by the addition of ATP. The concentrations of CPZ given in the text are the final concentrations of the drug in the reaction systems.

* Hexokinase (crystalline), Pabst laboratories. Glucose-6-PO_4 dehydrogenase, Type II, Sigma Chemical Co., St. Louis, Mo.

The reaction in the absence of CPZ was first studied to determine the experimental conditions to be used in the studies with CPZ and as a check on the characteristics of the enzyme preparation used. With glucose (0·1 M) and $MgCl_2$ (1×10^{-3}M) held constant, ATP was a strong inhibitor of the reaction when present in excess of $2{\cdot}5 \times 10^{-4}$M (Fig. 2). When glucose and ATP were held constant, inhibition was observed if the magnesium concentration greatly exceeded the initial ATP concentration (Fig. 3). A number of reports indicate molar ratios of Mg–ATP near 1:1 as the true sub-

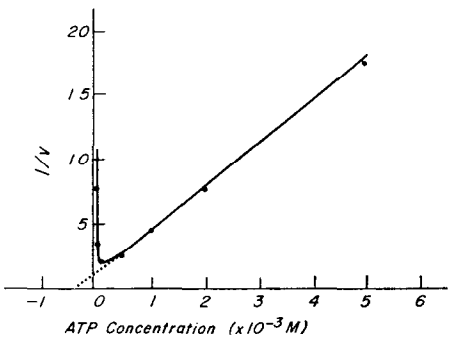


FIG. 2. Effect of ATP concentration on initial reaction rates in the presence of 1×10^{-3}M $MgCl_2$.

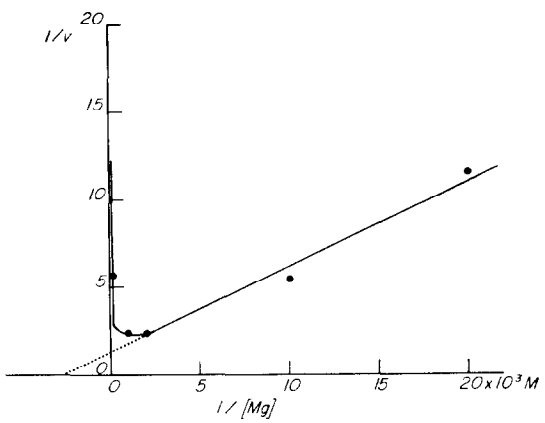


FIG. 3. Reciprocal plot of the effect of Mg^{2+} concentration on initial reaction rates in the presence of 1×10^{-3}M ATP.

strate species of phosphokinase reactions;[15–17] a plot of enzyme activity against Mg–ATP (1:1) concentration is given in Fig. 4. This 1:1 Mg–ATP ratio was used in all subsequent studies.

The effect of CPZ on the initial enzyme rates were assessed in a hexokinase system in which half-maximum activity was obtained at a Mg–ATP concentration of $2{\cdot}5 \times 10^{-4}$M The maximum initial velocity was found to correspond to a change in absorbancy of 0·100 units/min.

Hexokinase activities at various concentrations of Mg–ATP were determined in the presence of four levels of CPZ: $8{\cdot}5 \times 10^{-6}$M, $1{\cdot}75 \times 10^{-5}$M, $3{\cdot}5 \times 10^{-5}$M, and $6{\cdot}8 \times 10^{-5}$M (Figs. 5–8). The enzyme activities at the various Mg–ATP concentrations in the absence of CPZ served as control rates.

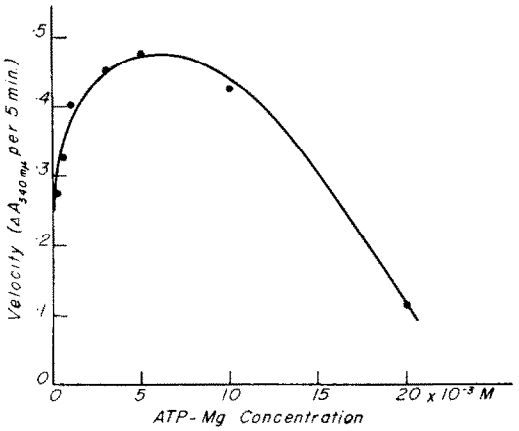
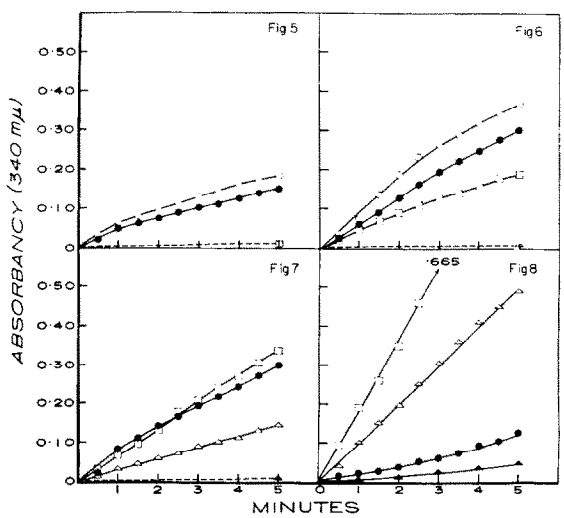


FIG. 4. Effect of ATP–Mg concentration at a 1 : 1 molar ratio on reaction rates.

RESULTS

CPZ acted as either an activator or inhibitor of hexokinase, depending on the concentration of the drug in relation to the concentration of Mg–ATP present. At the lowest concentration of CPZ ($8{\cdot}5 \times 10^{-6}$M), a slight activating effect on hexokinase was observed with a Mg–ATP concentration of 1×10^{-4}M, while at a higher concentration of Mg–ATP (5×10^{-4}M), a 35 per cent increase over the control rate of hexokinase activity was observed.

In the presence of $1{\cdot}75 \times 10^{-5}$M CPZ, no measurable hexokinase activity was detected with a Mg–ATP concentration of 1×10^{-4}M; however, by increasing the



FIGS. 5–8. The effect of chlorpromazine–HCl on yeast hexokinase. Mg-ATP (1 : 1 M). Conc.: Fig. 5, 1×10^{-4} M; Fig. 6, 5×10^{-4} M; Fig. 7, 1×10^{-3} M; Fig. 8, 2×10^{-3} M. Chlorpromazine-HCl concentration:

(●) none (control); (○) $8{\cdot}5 \times 10^{-6}$ M;
(□) $1{\cdot}75 \times 10^{-5}$ M; (△) $3{\cdot}5 \times 10^{-5}$ M;
(▲) $6{\cdot}8 \times 10^{-5}$ M.

Mg–ATP concentration, it was possible to restore the kinase activity to 70 per cent of the control rate with 5×10^{-4}M Mg–ATP and to approximately 100 per cent of the control rate with 1×10^{-3}M Mg–ATP. When the Mg–ATP concentration was raised to 2×10^{-2}M, the presence of $1{\cdot}75 \times 10^{-5}$M CPZ increased the enzyme activity 4·5 times over that of the control rate observed at this level of Mg–ATP.

At $3{\cdot}5 \times 10^{-5}$M CPZ, no measurable hexokinase activity was found with a Mg–ATP concentration of 5×10^{-4}M; however, enzyme activity could be restored by higher concentrations of Mg–ATP. A concentration of 1×10^{-3}M Mg–ATP restored

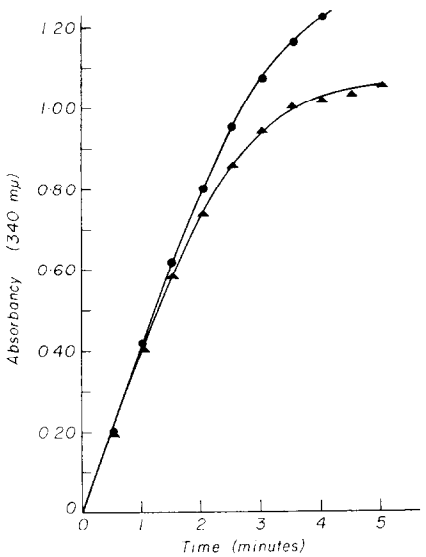


FIG. 9. Effect of CPZ on glucose-6-phosphate dehydrogenase.
(●) no CPZ; (▲) $6{\cdot}8 \times 10^{-5}$ M CPZ.

the enzyme activity to 30 per cent of the control rate. However, in the presence of this level of CPZ, a twofold increase in rate over that of control was observed when the Mg–ATP concentration was raised to 2×10^{-2}M.

At the highest concentration of CPZ used in these experiments ($6{\cdot}8 \times 10^{-5}$M) no hexokinase activity was found with concentrations of Mg–ATP up to 5×10^{-3}M (not shown in figure). An increase of Mg–ATP to 2×10^{-2}M restored the rate to only to 10 per cent of that of the control.

Since the reaction rate in the presence of the highest level of CPZ used in these experiments ($6{\cdot}8 \times 10^{-5}$M) was restored to only 10 per cent of the control rate by 2×10^{-2}M Mg–ATP, it was important to determine whether the almost complete loss of enzyme activity observed at this concentration of CPZ was the result of the action of the drug on hexokinase or the result of a rate-limiting inhibition of the auxiliary enzyme. To determine the activity of the *Zwischenferment* alone, an excess of glucose-6-phosphate was substituted for Mg–ATP in the reaction system. The rate of TPN-reduction, under these conditions, indicated that the *Zwischenferment* activity, in the presence of $6{\cdot}8 \times 10^{-5}$M CPZ, was inhibited by only 20 per cent, and therefore did not limit the rate of the reaction sequence (Fig 9).

DISCUSSION

These studies suggest that small variations in the concentration of CPZ can either activate or inhibit activity of an isolated hexokinase system. When the Mg–ATP concentration is low in comparison to the concentration of CPZ, the drug acts essentially as an inhibitor of this kinase system and prevents the utilization of ATP; whereas, at high concentrations of Mg–ATP relative to the concentration of CPZ, the drug functions as an activator, increasing the rate of utilization of ATP.

This apparent dual activity of CPZ has been observed in more complex enzyme systems and in tissue preparations, in which stimulatory effects have been observed at low concentrations of CPZ, while at higher concentrations of the drug, depressed activity was obtained.[8, 11, 18, 19] As an example, in rat brain homogenates, glutamine synthesis was stimulated by low concentrations of CPZ, while at only slightly higher concentrations the drug abruptly inhibited glutamine formation by nearly 100 per cent.[19] A biphasic action of CPZ on the DNP-stimulated ATPase reaction in rat-liver mitochondria has recently been reported by Löw,[20] who, in addition, found that this effect is apparently not restricted to CPZ; a number of chemically-unrelated substances, such as atebrin, acriflavin and antimycin A, also exhibit a similar effect on DNP-stimulated ATPase.[21] It seems pertinent to our observations in the isolated enzyme system that a similar dual effect has been observed in whole organisms. Low concentrations of CPZ have been shown to stimulate the growth of *L. acidophilus*, whereas high concentrations of the drug inhibited growth of this organism,[22] and growth of vitamin B_6-deficient rats has been stimulated by low doses of CPZ, while higher levels either failed to stimulate or depressed growth.[23]

The observed effects of CPZ on yeast hexokinase reported here resemble those described by Yanagawa[24] who reported stimulation of rat brain hexokinase by low levels of CPZ but inhibition by higher levels, and by Allenby and Collier[25] who found various phenothiazines to be potent inhibitors of rat brain hexokinase. However, there are several reported studies of the effect of CPZ on anaerobic glycolysis which differ from these results. Löw[26] investigated the effect of CPZ on yeast hexokinase by determining the decrease in acid-labile phosphate and observed no difference in ATP utilization in the presence of CPZ. Bernsohn *et al.*[9] reported that 1×10^{-3}M CPZ had no effect on anaerobic glycolysis in rat brain homogenates. However, under aerobic conditions he found that CPZ greatly depressed the utilization of glucose by the homogenates but not by isolated yeast hexokinase. A possible explanation for the differences between the results of the above investigators[9, 26] and those reported here may be found in differences in concentrations and ratios of ATP and magnesium chosen and in the methods used by the various investigators. Indeed, one factor which seems to stand out in the work reported here is that CPZ appears to affect the "dissociation" or "binding" constants of the enzyme for Mg and ATP, which have already been shown to be interdependent cofactors of hexokinase. Therefore, any conclusions projected from observed results in this system are closely dependent on the choice of experimental conditions.

The results presented in this paper suggest that the often observed hyperglycemic effect following the administration of CPZ should be accompanied by an accumulation of ATP if its utilization were decreased. This contention is supported by the studies of Grenell[27] who found increased levels of ATP in the lungs and cerebral cortex of CPZ-treated rats. In nervous tissues, the greatest increase in ATP was observed in the

thalamus–hypothalamus area of the brain. These increased tissue levels of ATP parallel the distribution patterns of tissue-CPZ reported by other investigators.[28, 29]

These observations are not sufficient to permit a definitive conclusion concerning the mechanism of action of the drug; however, our data seem to implicate interference with utilization of ATP as a fundamental metabolic effect of chlorpromazine administration.

SUMMARY

Chlorpromazine may either inhibit or activate the utilization of ATP by an isolated system of yeast hexokinase. This biphasic action of the drug appears to be a function of the relative concentrations of the drug and the Mg-ATP substrate. The ability of chlorpromazine either to stimulate or to inhibit the utilization of ATP suggests that it may function as a metabolic regulator. The possibility of attributing some of the pharmacologic properties of the drug to its apparent regulatory action is discussed.

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