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KINETIC MECHANISM OF CHLORPROMAZINE INHIBITION OF ERYTHROCYTE 3-*O*-METHYLGLUCOSE TRANSPORT

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The kinetic mechanism of chlorpromazine inhibition of erythrocyte hexose transport was investigated using the non-metabolizable glucose analog 3-*O*-methylglucose. It was found that chlorpromazine added to the external medium is a non-competitive inhibitor of both equilibrium exchange and net 3-*O*-methylglucose transport at pH 7.8, 15°C. The K_i for equilibrium exchange is 76 ± 21 μM. When net efflux and equilibrium exchange were measured on the same population of cells the equilibrium exchange was 2.5-times the maximum net efflux. The percent reduction of 3-*O*-methylglucose flux by chlorpromazine is dependent upon chlorpromazine concentration and not 3-*O*-methylglucose concentration as expected for a non-competitive inhibitor. Equilibrium exchange and net efflux show the same extent of inhibition at each concentration of chlorpromazine evaluated. These results suggest that exchange and net efflux of 3-*O*-methylglucose in the human erythrocyte may share a common transport system.

The effect of phenothiazines on erythrocyte morphology and transport systems has been extensively studied. Phenothiazines, notably chlorpromazine, have been shown to cause membrane shape change at low concentrations, hemolysis at high concentrations [1,2], and to inhibit both glucose transport and chloride transport [3–5]. Although the mechanism of its membrane effects are not known, chlorpromazine is highly lipophilic and has been shown to incorporate into the plasma membrane [6]. Two laboratories have investigated the effect of chlorpromazine on erythrocyte hexose transport and have reported that chlorpromazine preferentially inhibits net transport while having no effect on exchange transport [4,5]. On this basis it was concluded that there is a fundamental difference between exchange and net flux pathways. These findings also suggest that chlorpromazine could be an extremely useful tool in the investigation of the kinetic mechanism of hexose transport, since the net component could be selectively eliminated. Therefore, in the present study chlorpromazine was evaluated as an inhibitor of equilibrium exchange and net hexose transport using the non-metabolizable glucose analog 3-*O*-methylglucose [7,8].

Blood was collected and cells were washed as described by Gunn and Fröhlich [9]. In the present experiments, cells were kept at 15°C and resuspended in a medium containing different concentrations of 3-*O*-methylglucose (2, 25 or 70 mM) (3-*O*-methyl-D-glucopyranose, Sigma Chemical Co., St. Louis, MO). Prior to the final wash 3-*O*-[^{3}H]methylglucose (75 μCi/mmol, New England Nuclear, Boston, MA) was added and allowed to

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equilibrate. Cells were packed by centrifugation in nylon tubes as previously described [9]. The efflux rate coefficient was measured by injecting packed cells into a well stirred thermostatically controlled tracer free solution of the same composition as extracellular fluid (equilibrium exchange flux) or the same composition without 3-*O*-methylglucose (net efflux). The supernatant fluid was sampled by rapid filtration at known time intervals (approximately a 12 s time course) after injection of the cells; a final sample was taken by centrifugation after 1 h at room temperature. The initial rate coefficient for equilibrium exchange and net efflux was determined as described in Ref. 9. Chlorpromazine was obtained from Sigma Chemical Co., St. Louis, MO. It was found to be 99% pure by mass spectral analysis.

When the effect of chlorpromazine on erythrocyte 3-*O*-methylglucose net efflux was measured it was found that the extent of inhibition of flux for 25, 50 or 75 μM chlorpromazine was 19.3, 39.7 and 49.5%, respectively (data not shown). Inhibition was independent of substrate concentration suggesting that chlorpromazine is a non-competitive inhibitor. Non-competitive inhibition was confirmed in separate experiments using a Dixon plot (1/flux vs. chlorpromazine concentration; Ref. 10) and the K_i was determined to be 75 ± 21 μM (Fig. 1). A similar K_i value was calculated when the data from Fig. 1 was plotted in a Cornish-Bowden plot (3-*O*-methylglucose concentration/Flux vs. chlorpromazine concentration; Ref. 11). This K_i value agrees well with the K_i reported by Motais et al. [5] of 80 μM but is markedly higher than the K_i of 2.5 μM reported by Baker and Rogers [4].

Although chlorpromazine had been previously reported not to inhibit erythrocyte glucose equilibrium exchange; this was not found to be true. When the effect of chlorpromazine on erythrocyte 3-*O*-methylglycose equilibrium exchange was measured, it was observed that chlorpromazine was also a non-competitive inhibitor of this process. Furthermore the degree of inhibition at 25, 50 and 75 μM chlorpromazine was 17.0, 36.0 and 43.2%, respectively (data not shown). Equilibrium exchange was found to equal 2.5 × maximal net efflux which agrees with the ratio of 2.5–3.0 reported by Mawe and Hempling [12]. The Dixon

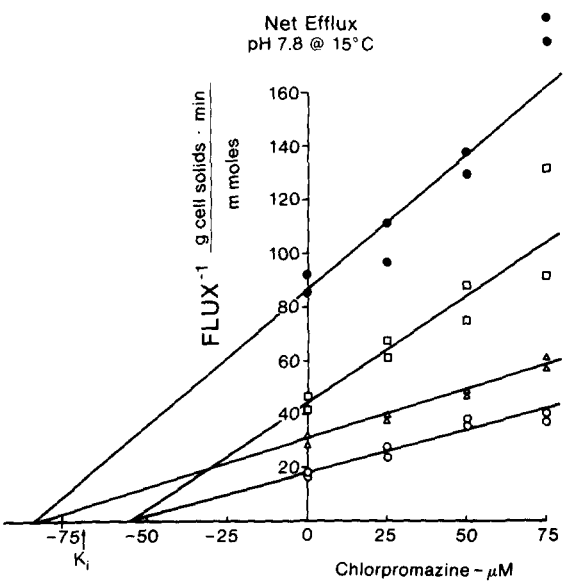

Fig. 1. Effect of chlorpromazine on erythrocyte 3-*O*-methylglucose net efflux. Dixon plot. pH 7.8 at 15°C. Cells were preloaded with 3-*O*-methylglucose (●, 2 mM; □, 5 mM; △, 10 mM; ○, 25 mM) and net efflux was determined by measuring efflux of 3-*O*-[^{3}H]methylglucose into tracer free and 3-*O*-methylglucose free medium. Efflux was evaluated in the presence of ethanol (control) or chlorpromazine (added from a 10^{-2} M ethanol stock). Values represent the mean of two quadruplicate determinations.

plot of a separate set of experiments (shown in Fig. 2) demonstrates that the K_i for equilibrium exchange is roughly equal to the K_i for net efflux, i.e. 76 ± 18 μM. When chlorpromazine inhibition data for exchange was plotted in a Cornish-Bowden plot a similar K_i was calculated.

The finding that the anionic phenothiazine chlorpromazine inhibits both net and equilibrium exchange erythrocyte hexose transport, refutes the claims of Baker and Rogers [3,4] and Motais et al. [5]. The difference between the present studies and those previously reported is largely one of experimental design. First, in the previous studies there were methodological differences in measurement of net and exchange transport. Both groups measured equilibrium exchange using radioactive tracer and net transport by an optical technique. In our studies, the rate constants for net and exchange were determined from tracer efflux measurements which had a very rapid time resolution and are actual initial rate constants determined over an

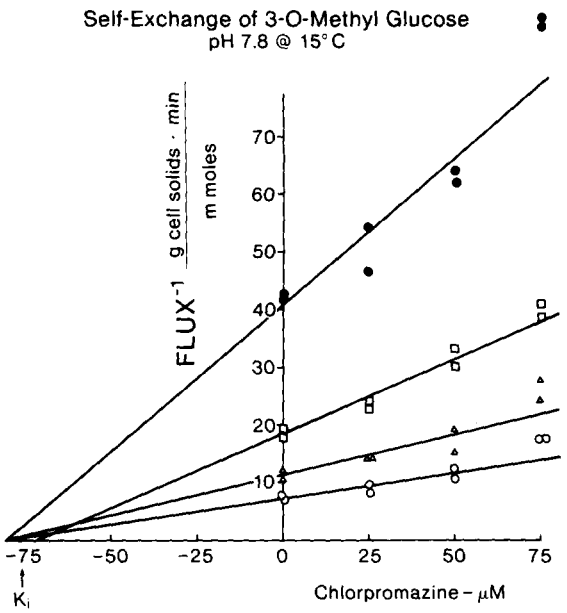

Fig. 2. Effect of chlorpromazine on erythrocyte 3-*O*-methylglucose equilibrium self-exchange. Dixon plot. pH 7.8 at 15°C. Cells were preloaded with 3-*O*-methylglucose and self-exchange was determined by measuring efflux of 3-*O*-[^{3}H]methylglucose into tracer free medium containing identical concentrations of 3-*O*-methylglucose (●, 2 mM; □, 5 mM; △, 10 mM; ○, 25 mM). Efflux was evaluated in the presence of ethanol (control) or chlorpromazine (added from a 10^{-2} M ethanol stock). Values represent the mean of two quadruplicate determinations.

efflux period of approx. 12 s. A second difference is the use of the nonmetabolizable glucose analog, 3-*O*-methylglucose versus glucose itself. Although 3-*O*-methylglucose has been shown to have transport characteristics identical with those of glucose [7,8], glucose metabolites and particularly phosphorylated intermediates may not. This could lead to difficulties in interpretation of data. Finally, the present measurements were made at 15°C for both exchange and net transport, while Baker and Rogers made their measurements at 17°C and Motais et al. measured net transport at 25°C and exchange transport at 0°C. Since glucose transport is extremely temperature sensitive (e.g., maximal exchange flux drops 8-fold from 25°C to 5°C [13]), it would be difficult to estimate initial rate constants at 0°C since transport is very slow. While the reason for the discrepancy between our results and the previously reported findings is not entirely clear, we feel it is unwarranted to compare inhibition data determined optically at 25°C with inhibition data determined with a radioactive tracer at 0°C. It is possible that the selective inhibition of erythrocyte hexose net transport previously reported [4,5] was due to experimental design and not to an actual phenomenological difference in the two transport processes.

Various kinetic models have been proposed for erythrocyte hexose transport, e.g. the symmetric carrier model [14], the tetramer model [15], the introverting hemiport model [16] and the allosteric pore model [17]. With each of these models, one of the most difficult experimental observations to reconcile has been the differences between the $K_{1/2}$ values for equilibrium exchange and net transport. In this regard, one explanation put forth was that there were separate systems for exchange and net transport. This contention was accordingly supported by the studies which suggested that chlorpromazine preferentially blocked net transport. However the present results indicate that the effect of chlorpromazine on net and equilibrium exchange hexose transport cannot be used as evidence for two transport systems. In fact, the chlorpromazine data presented above suggest that the two processes may share a common carrier. It is also worth noting that Zipper and Mawe using radioactive tracer technique to measure both net and exchange glucose flux, observed that chlormedrin, a sulfhydryl blocking agent, affected net and exchange efflux to the same extent [18]. However they also found that *p*-chloromercuribenzene sulfonate and insulin had differential effects on net and exchange transport [19]. Other studies using the inhibitor cytochalasin b have indicated that its differential effects on net and exchange flux could be attributed to asymmetric binding to the transport protein [20].

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