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EFFECTS OF PSYCHOTROPIC DRUGS ON THE ERYTHROCYTE PERMEABILITY TO GLUCOSE AND ETHYLIDENE GLUCOSE

G. F. BAKER and H. J. ROGERS

Department of Physiology, Bedford College, London, N.W.1 and Department of Pharmacology, Guy's Hospital Medical School, London, S.E.1

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Abstract—The effect of chlorpromazine (CPZ) and a number of CNS depressant drugs on glucose penetration through human erythrocyte membrane has been investigated by an optical technique.

CPZ accelerated glucose exit at concentrations between 1×10^{-5} and 2×10^{-5} M at 36° but at higher concentrations inhibited transfer. This inhibition was rapidly and completely reversible. CPZ, trifluoperazine, prochlorperazine, promazine and promethazine were found to inhibit glucose exit approximately in the order of their chemotherapeutic potency. Imipramine also showed this effect but nealbarbitone, thiopentone and haloperidol did not.

CPZ affects the entry of glucose into erythrocytes in a biphasic manner similar to its effect on exit but at all concentrations it accelerates the penetration of ethylidene glucose which enters by diffusion.

CPZ had no effect on the inhibition of glucose transfer produced by incubation with dinitrofluorobenzene (DNFB) or on the enhancement of this inhibition by incubation in the presence of glucose and 2-deoxyglucose.

It is suggested that at low concentrations CPZ accelerates the movement of the glucose carrier within the membrane by effects on its charge environment. At higher concentrations interaction with the protein of both membrane and carrier presumably causes interference with carrier movement until at high concentrations haemolysis occurs. The relevance of these effects to the pharmacological action of CPZ is discussed.

THE EXISTENCE of facilitated diffusion of glucose from blood to brain[1–3] and blood to C.S.F.[4] has been established by experiments *in vivo* and a similar carrier mediated transport of sugars has been shown in brain slices.[5] The passage of glucose across the neuronal membrane may exert an important control over brain metabolism.[6] Alterations in glucose penetration have been postulated to explain both chlorpromazine induced hyperglycaemia[7] and the increased brain glucose concentration during anaesthesia and chlorpromazine treatment.[8] At present it is not possible to investigate the kinetics of glucose transfer and its modification by drugs in cerebral tissues using direct methods. The general properties of the system with respect to saturability, stereospecificity, affinity for various sugars and lack of effect of insulin and metabolic inhibitors appear to be similar to those found in the erythrocyte. The recent estimation by Bachelard[5] of a half-saturation constant, K_m, for glucose of the order of 5 mM in brain slices is comparable with that found for the erythrocyte.[9] In view of these findings and the amount of information available on both the membrane stabilizing effects of psychotropic drugs and on the kinetics of glucose penetration into the red cell, it was considered useful to investigate the effect of some psychotropic drugs, in particular chlorpromazine (CPZ), on the glucose transfer system of the erythrocyte.

METHODS

Blood was collected by venepuncture, heparinized and the cells washed three times in buffered saline before use. Blood was used up to 72 hr after collection without effect on the congruence of the results. The exit and entry of glucose and the entry of 4,6-0-ethylidene-D-glucopyranose (Koch–Light), "ethylidene glucose", were followed in an Ørskov-type photoelectric apparatus and the results analysed as detailed in Widdas[10] and Sen *et al.*[9] 2,4-Dinitro-l-fluorobenzene (B.D.H.)—"DNFB", was prepared and used as detailed by Bowyer *et al.*[11] Chlorpromazine HCl (May and Baker), nealbarbitone (May and Baker), thiopentone sodium (May and Baker), trifluoperazine HCl (Smith, Kline and French), imipramine HCl (Biorex), promazine HCl (Wyeth) and haloperidol (Roche) were studied. Drug solutions were prepared fresh before each experiment in a medium buffered at pH 7·2 containing Na^+ 155·8 mmole/l; K^+ 5·6 mmole/l; Ca^{2+} 4·3 mmole/l; Cl^- 163·9 mmole/l and HCO_3^- 1·8 mmole/l and these solutions were used as the suspending medium during both exit and entry experiments. In trial experiments it was found that preincubation in drug solutions gave results identical with those in which the preincubation had been in saline medium, the latter method was therefore more usually used. CPZ *per se* was found not to alter red cell volume under the conditions of these experiments.

RESULTS

Effects on glucose permeability. The effect of various concentrations of CPZ on the exit of glucose from erythrocytes previously loaded with 76 mM glucose was investigated. The results of experiments conducted at 36° are shown in Fig. 1 in which exit time is plotted against CPZ concentration. Since under these experimental conditions exit time is inversely related to the transfer rate such a plot is a modification of the Dixon plot used in enzymology and should be a straight line if inhibition follows Michaelis–Menten kinetics. As can be seen this is not the case: after an initial fluctua-

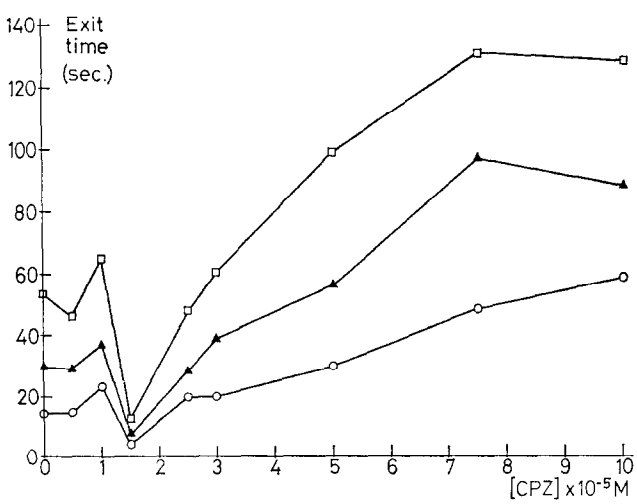

FIG. 1. The effect of chlorpromazine on the exit time of 76 mM glucose from human erythrocytes. The points represent the mean of between 3–12 determinations. External glucose concentrations: ○, 0·7 mM (Control); ▲, 4·5 mM; □, 10·1 mM.

tion the exit time drops sharply to about a third of its control value before rising in a non-linear manner and levelling off at around three times control value.

The same response was observed at 27°, 22°, and 17°, but with lower temperature the point of inflexion was found to occur at a higher concentration, e.g. it is 3×10^{-5} M at 22°, and the biphasic shape of the curve was emphasized. It is interesting that the CPZ concentration at which saturation of membrane stabilizing sites occurs was found to be approximately 2×10^{-5} M at 22° by Kwant and Seeman.[12]

Glucose entry at 36° showed similar changes in rate to those seen with the exits at that temperature, the point of inflexion lying between 1×10^{-5} and $2{\cdot}5 \times 10^{-5}$ M CPZ.

The influence of glucose in the external medium on exit at 36° is shown in Fig. 2 (Sen–Widdas plot). In such plots the intercept on the abscissa is equal to $-K_m$, the half saturation constant, and that on the ordinate is $1/V_{max}$, the reciprocal of maximum velocity. This also reflects the anomalous effect of CPZ on the velocity of glucose

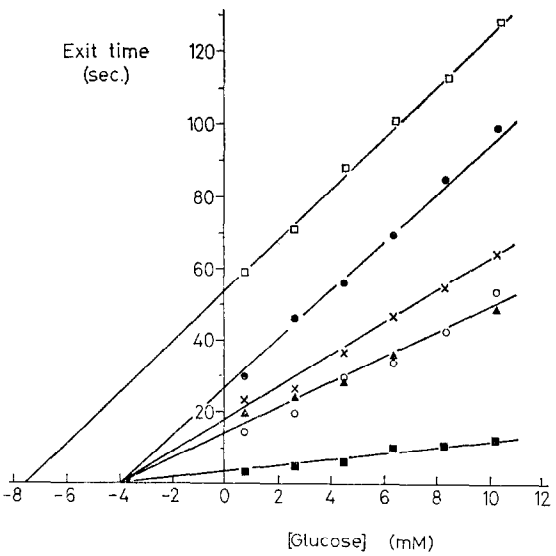

FIG. 2. Sen–Widdas plot of the exit times of 76 mM glucose from human erythrocytes into glucose solution containing chlorpromazine. Chlorpromazine concentrations: ○, Control; ×, $1{\cdot}0 \times 10^{-5}$ M; ■, $1{\cdot}5 \times 10^{-5}$ M; ▲, $2{\cdot}5 \times 10^{-5}$ M, ●, 5×10^{-5} M; □, $1{\cdot}0 \times 10^{-4}$ M.

transfer but shows that over the lower range of concentrations the half-saturation constant is unaffected. This indicates that the effect of the drug is non-competitive and that it is acting elsewhere than at the site of glucose binding. The alteration of the half-saturation constant by 1×10^{-4} M CPZ suggests that the binding site has become affected by the CPZ at this high concentration but this may be part of a progressive membrane involvement which leads to haemolysis at concentrations of this order.

These drug effects on the exit and entry of glucose were found to be completely and rapidly reversible upon washing the cells at all sub-lytic concentrations of CPZ.

The results of experiments with 1·4 mM DNFB support the findings that CPZ (10^{-5} to 10^{-4} M) does not interfere with the glucose binding site. CPZ exerts no influence on the irreversible inhibition of glucose transfer produced by DNFB nor does

it affect the enhancement of the development of inhibition by incubation with DNFB in the presence of glucose or 2-deoxyglucose. This latter effect has been interpreted as the stabilization by transported sugars of a conformation of the carrier which is favourable to DNFB combination and this conformational state of the carrier with its bound glucose is postulated to be an intermediate in the transport process.[13] This behaviour is different from that of some other substances: mainly detergents such as Triton X-100 and sodium lauryl sulphate, which have membrane stabilizing properties[14] and inhibit glucose penetration in a non-competitive manner but which also accelerate the development of DNFB inhibition.[15]

Effects on ethylidene glucose permeability. Because of the complex nature of the CPZ action on glucose permeability it was decided to study its effects on the permeability of a substance which penetrates the red cell by diffusion. Ethylidene glucose is a glucose derivative which appears to penetrate in such a manner[16] and its slow rate of penetration makes it suitable for study by the Ørskov technique. Fig. 3 shows the effect of CPZ

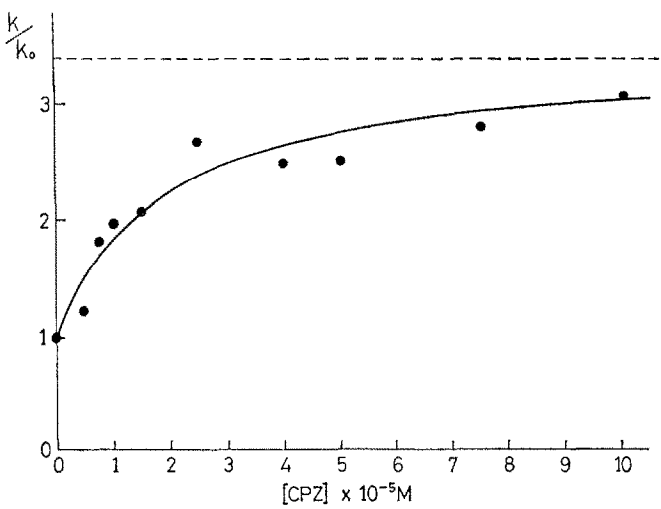

FIG. 3. The rate of diffusion of 38 mM ethylidene glucose into human erythrocytes in the presence of chlorpromazine relative to the rate in its absence. The points are the mean of between 2–5 observations. The solid line is drawn according to Eq. (2) using the parameters from Fig. 4. The broken line (asymptote) represents the theoretical maximum ratio.

on the diffusion rate constant, k, of ethylidene glucose measured from F(C.V.) plots as described by Widdas.[10] The results have been normalized since there is some variation between individuals.

The rate constant for diffusion, k, is in isotonic units min^{-1} and can be converted to, P, the permeability constant per second using the equation

$$P = \frac{k}{60} \times \frac{\text{cell vol.}}{\text{cell area}} = k \times 0{\cdot}83 \times 10^{-6}.$$

If it is assumed that the cell membrane is made up of a large number of discrete regions with a normal diffusion coefficient D and that there are [M] sites per cm^2

capable of adsorbing CPZ which then alters the diffusion coefficient in the vicinity to D′, it may be assumed that the permeability in the presence of the drug will be given by

$$P = \mathrm{D}([\mathrm{M}] - [\mathrm{MA}]) + \mathrm{D}'[\mathrm{MA}] \tag{1}$$

where [MA] is the concentration of altered sites.

Assuming the reaction of CPZ with the membrane sites follows normal kinetics the affinity constant, K_a, will be given by

$$K_a = \frac{[\mathrm{A}]\,([\mathrm{M}] - [\mathrm{MA}])}{[\mathrm{MA}]},$$

where [A] is the CPZ concentration, whence

$$[\mathrm{MA}] = \frac{[\mathrm{M}]\,[\mathrm{A}]}{[\mathrm{A}] + K_a},$$

Substituting in (1) for [MA] gives

$$P = \mathrm{D}[\mathrm{M}] - \frac{\mathrm{D}[\mathrm{M}]\,[\mathrm{A}]}{[\mathrm{A}] + K_a} + \frac{\mathrm{D}'[\mathrm{M}]\,[\mathrm{A}]}{[\mathrm{A}] + K_a}.$$

Rearranging

$$P = \mathrm{D}[\mathrm{M}]\left(1 - \frac{[\mathrm{A}]}{[\mathrm{A}] + K_a} + \frac{\mathrm{D}'[\mathrm{A}]}{\mathrm{D}[\mathrm{A}] + K_a}\right).$$

Now D[M] is the unaffected permeability which is proportional to the control rate constant, so that;

$$\frac{k}{k_0} = 1 + \left(\frac{\mathrm{D}'}{\mathrm{D}} - 1\right)\frac{[\mathrm{A}]}{[\mathrm{A}] + K_a}. \tag{2}$$

and

$$[\mathrm{A}] = \left(\frac{\mathrm{D}'}{\mathrm{D}} - 1\right)\frac{[\mathrm{A}]}{\dfrac{k}{k_0} - 1} - K_a. \tag{3}$$

Thus plotting

$$\frac{[\mathrm{A}]}{\dfrac{k}{k_0} - 1}$$

against [A] should generate a straight line of slope

$$\frac{1}{\dfrac{\mathrm{D}'}{\mathrm{D}} - 1}$$

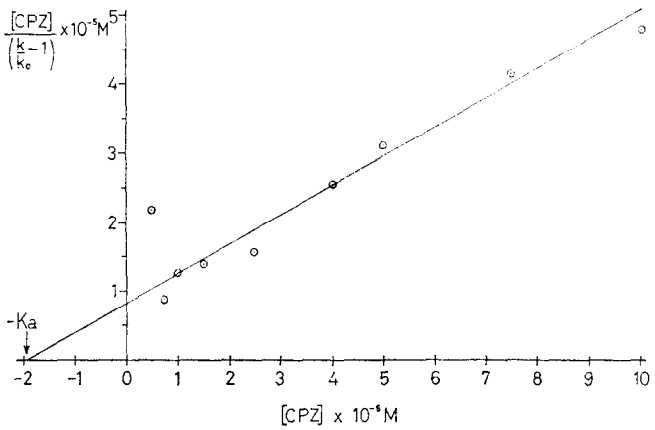

FIG. 4. Data from ethylidene glucose experiments plotted according to Eq. (3). $K_a = 1{\cdot}95 \times 10^{-5}$ M; $D'/D = 3{\cdot}4$. Line fitted by least squares method.

and intercept $-K_a$. This is Fig. 4 and from it K_a is found to be approximately $1{\cdot}95 \times 10^{-5}$ M at 36°.

DISCUSSION

It would seem from these results that CPZ facilitates the passage of molecules such as ethylidene glucose across the membrane and the uptake of drug by the membrane follows, at least as far as this effect is concerned, classic kinetic theory.

It has been shown that CPZ is adsorbed by sites in erythrocyte ghost membranes and that this process is associated with expansion of the membrane and stabilization against hypotonic haemolysis.[17] Adsorption at these stabilizing sites is complete at around 2×10^{-5} and above this the membrane concentration rises steeply. In our experiments there was no evidence of an abrupt change of ethylidene glucose permeability even approaching concentrations that caused spontaneous haemolysis. The affinity constant found by Kwant and Seeman[12] was 6×10^{-6} M at 22°. This may be compared with the present value of $1{\cdot}95 \times 10^{-5}$ M obtained at 36° with intact red cells.

The inhibitory effect on glucose transfer seen at higher concentrations was exhibited by all the phenothiazine derivatives examined. Trifluoperazine was more effective than CPZ, prochlorperazine and promazine which were more effective than promethazine. Owing to the complex nature of the inhibition it is difficult to assign an exact order of potency to these compounds. The related antidepressant compound imipramine also had inhibitory effects intermediate between those of CPZ and trifluoperazine. Nealbarbitone, thiopentone sodium and haloperidol showed no inhibitory effects in the concentration range studied (10^{-7} to 10^{-3} M for barbiturates, 10^{-8} to 10^{-5} M for haloperidol).

The action of CPZ on the transport of glucose across the erythrocyte membrane shows a complex behaviour which must be due to the simultaneous effect of several different actions, the actual degree of any effect being dependent on the concentration. An attempt to explain the changes observed must, therefore, be based on actions which are known to occur at corresponding concentrations.

The initial increase and decrease in rate occur in a concentration range found by Kwant and Seeman[18] to cause the displacement of membrane bound calcium from erythrocyte ghosts. Certainly in our experiments low CPZ concentrations were found to cause clumping of the cells which would be an indication of change in surface charge. One of the functions of calcium in the membrane may be the maintenance of membrane structure, thus loss of calcium would result in a rearrangement of the membrane components and, possibly an alteration of surface charge with a resultant change in transfer rate.

The sharp reduction in exit time seen above 1×10^{-5} M is difficult to account for fully. If, as the results for ethylidene glucose suggest, CPZ alters the membrane structure so that the diffusion of molecules occurs more rapidly the glucose carrier may also be speeded up but this effect is unlikely to be sufficient to explain the three-fold increase in rate that is observed.

From this very high rate of transfer the system is progressively inhibited as the CPZ concentration rises further. As was seen from Fig. 2 this inhibition and the preceeding effects do not involve the glucose-carrier binding site but probably involve a change in the tertiary structure of the carrier or perhaps the surface protein of the membrane.

At still higher concentrations the change progresses to include the binding site, resulting in a decrease in affinity of the carrier for glucose accounting for the increased value of the half-saturation constant.

The acceleration of ethylidene glucose penetration is probably related to the expansion of the cell membrane by CPZ. This may be either a direct effect on membrane lipids[19] or some change resulting from the interaction of CPZ with the surface protein which causes the lipids to be exposed at the membrane surface. Such changes would continue as the concentration increased until finally drug interaction with the membrane protein and lipid produced disruption of the membrane and lysis of the cell.

Many actions of CPZ demonstrate a biphasic effect, low concentrations producing the opposite effect to high ones.[20] CPZ has been shown to inhibit glucose entry into isolated rat spinal cord and muscle[21] whilst experiments in mice have shown an increase in brain glucose which may be explicable in terms of an increased glucose transfer rate across neuronal membranes.[8] These conflicting observations may perhaps be reconciled by our observations. An inhibitory affect on glucose penetration through membranes has been demonstrated in the case of some other C.N.S. depressants, butanol has been shown to inhibit glucose transfer in red cell by optical[22] and isotope[23] techniques although haematocrit methods have failed to confirm this.[24] The inhalation anaesthetics ether, halothane, and methoxyflurane also inhibit glucose penetration into erythrocytes in therapeutic concentrations.[24]

The drugs studied here and exemplified by CPZ have a complex effect on red cell glucose permeability being acceleratory at low concentrations and inhibitory at higher concentrations. If the membranes of brain cell have similar properties the predominant effect must depend on local concentrations but in addition interpretations should bear in mind that brain cells will have a much greater range of surface to volume ratios than red cells and so a concentration of CPZ which was inhibitory would affect first those cells whose glucose utilization was at such a level as to be membrane limited. In this range such an inhibitory action could be contributory to the reduction in the general level of cerebral metabolism which occurs during sedation.

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