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Phlorizin–receptor interactions in fat cell plasma membranes

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SUMMARY

The rate but not the extent of phlorizin binding to purified fat cell plasma membranes was temperature dependent and this binding was a saturable process. A Scatchard plot revealed a population of sites which exhibited a dissociation constant of about 0.35 mM and a maximum binding capacity of about 8 nmoles/mg membrane protein. Under the conditions of these experiments neither glucose, phloretin, nor cytochalasin B inhibited [^{3}H]phlorizin binding. These data demonstrate the presence in fat cell plasma membranes of specific receptors for phlorizin which may mediate the inhibitory effects of this agent on hexose transport.

Phlorizin and its aglucone phloretin are known to inhibit membrane transport of monosaccharides and other substances in a large number of tissues studied[1–3]. Phlorizin blocks D-glucose uptake by active transport systems more effectively than phloretin whereas the reverse order of potency is observed with facilitated diffusion systems. In isolated fat cells, which exhibit the latter mode of glucose uptake[4], we found that half-maximal inhibition of glucose oxidation due to phloretin or phlorizin occurred at about 6 μM and 140 μM, respectively[5].

In facilitated diffusion systems, little is known about the interaction of these agents with specific cell membrane components. Information of this type is probably mandatory before the mechanisms involved in transport inhibition can be understood. LeFevre and Marshall[6] reported that phloretin bound extensively to red blood cells but saturation of specific sites could not be demonstrated. In this paper we report our studies on the binding of [^{3}H]phlorizin to fat cell plasma membranes which show that the rate of phlorizin binding to membranes is temperature dependent and that binding to specific receptors is saturable. Further, Scatchard analysis revealed a population of binding sites

exhibiting a dissociation constant of 0.35 mM which is close to the concentration of phlorizin which exhibits half-maximal inhibition of glucose transport[5] in intact fat cells.

White fat cells were obtained from parametrial adipose tissue (Charles River CD strain female rats) by enzymatic digestion with crude collagenase (*Clostridium histolyticum*, Worthington) in Krebs–Ringer phosphate buffer containing 3% bovine serum albumin as described previously[7]. Plasma membranes were purified on a discontinuous sucrose gradient according to a modification of the method of McKeel and Jarett[8] as described by Czech and Lynn[9]. Binding of [^{3}H]phlorizin (New England Nuclear, specific labeled sites unknown) to membranes was performed in triplicate by adding 20 μl fat cell membranes (20–40 μg protein) to 70 μl Krebs–Ringer phosphate buffer followed by addition of 10 μl [^{3}H]phlorizin (0.25–0.5 μCi per tube). The reactions were terminated by the addition of 2 ml of ice cold buffer to the incubation tubes and the contents decanted onto HA Millipore filters. The filters were washed twice with 4 ml of ice cold Krebs–Ringer phosphate buffer and the radioactivity retained was determined[5]. The washing procedure took about 15 s.

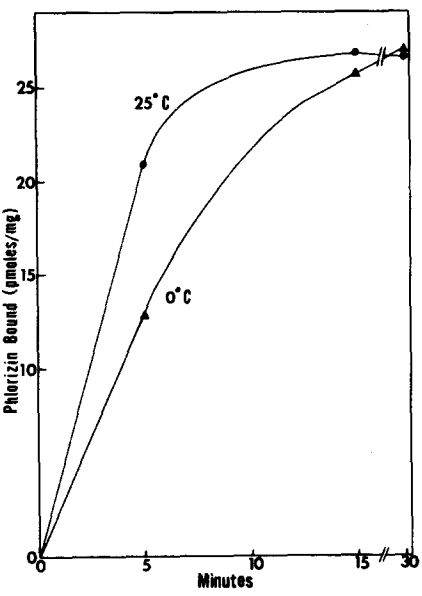
The values presented were corrected for nonspecific binding of label to filters by adding the ice cold buffer with the [^{3}H]phlorizin to control tubes and subtracting these zero time values from the total binding data obtained. Protein determinations were performed according to the method of Lowry *et al.*[10].

Fig. 1 shows the rate of [^{3}H]phlorizin uptake by purified fat cell plasma membranes at 0 °C and 25 °C. Binding was essentially complete by 10 min at 25 °C and by 30 min the amount of phlorizin bound was the same at both temperatures. This similarity in the extent of binding at 25 and 0 °C suggests the absence of significant enzymatic degradation of phlorizin in these membranes. In contrast, phlorizin hydrolase in intestinal brush border[11] catalyzes the rapid conversion of phlorizin to phloretin and prohibits phlorizin–receptor interaction studies in this tissue. Thus phlorizin binding to fat cell membranes can be studied at temperatures between 0 and 25 °C without measurable differences in the amount of phlorizin bound.

The results presented in Fig. 2 show the effect of increasing concentrations of unlabeled phlorizin on the binding of 10 μM [^{3}H]phlorizin. Unlabeled phlorizin markedly decreased the total label bound until at 5 mM unlabeled phlorizin over 85% inhibition was found. Higher concentrations of unlabeled phlorizin were not used because of incomplete solubility. Thus, [^{3}H]phlorizin binding to fat cell membranes as assayed by the methods described is a highly specific and saturable process.

The simplest interpretation of a Scatchard plot (Fig. 3) of the binding of various concentrations of [^{3}H]phlorizin (Fig. 2) indicates at least 2 populations of binding sites. The higher affinity site has an apparent K_m of about 0.35 mM and maximally binds about 8 nmoles phlorizin per mg membrane protein. The lower affinity site probably represents nonspecific binding and may not be saturable. It is interesting that the dissociation constant of the high affinity site is quite close to the concentration of phlorizin which inhibits fat cell glucose oxidation half-maximally[5].

We have previously reported that cytochalasin B, phloretin and phlorizin exhibited



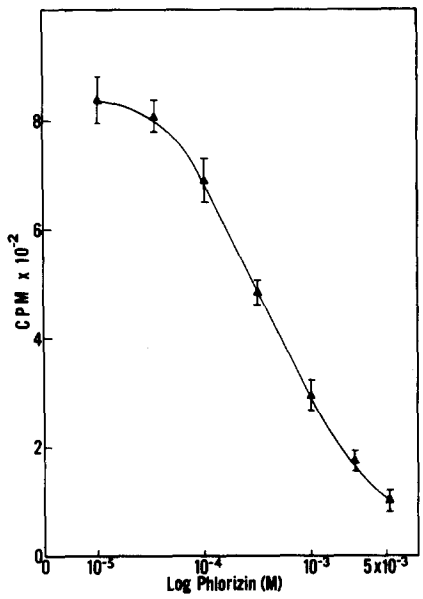


Fig. 1. Temperature dependence of phlorizin binding to isolated white fat cell plasma membranes. The membranes (32 μg protein/tube) were incubated in triplicate at 25 °C or 0 °C with 0.7 μM [^{3}H]phlorizin in Krebs–Ringer phosphate buffer to give a final volume of 0.1 ml. The reactions were stopped at the times indicated and the bound label was determined.

Fig. 2. The effect of varying concentrations of unlabeled phlorizin on the binding of [^{3}H]phlorizin to isolated fat cell plasma membranes. Membranes (21 μg protein) were incubated for 30 min at 25 °C in phosphate buffer in a final volume of 0.1 ml. Unlabeled phlorizin was added with the [^{3}H]phlorizin and the net radioactivity bound to the membranes was determined.

the same relative effectiveness in inhibiting fat cell glucose transport and alveolar macrophage phagocytosis[12]. This observation was consistent with the concept that these three agents interact with a membrane component common to several cell types. In the present studies we tested the ability of phloretin and cytochalasin B to inhibit [^{3}H]phlorizin binding to fat cell membranes. As shown in Table I no effect of 0.1 mM cytochalasin B or 0.3 mM phloretin was found and 1 mM phloretin actually increased binding slightly. Thus under the conditions of these studies no evidence suggesting common receptors for these agents was found.

In conclusion, the demonstration in this report of specific, saturable binding sites for phlorizin in fat cell plasma membranes suggests it may be possible to isolate membrane component(s) specific for phlorizin binding which may in turn provide insight into the inhibitory action of this agent on membrane transport processes. The failure of cytochalasin B or phloretin to bind at the same site as phlorizin suggests that many different binding sites which can alter hexose transport exist in fat cells.

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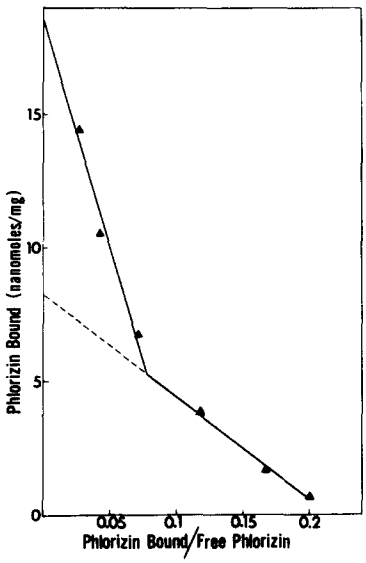

Fig. 3. Scatchard plot of phlorizin binding to isolated plasma membranes of white fat cells. The membranes (21 μg protein) were incubated at 25 °C for 30 min with varying concentrations of [^{3}H]phlorizin as in Fig. 2.

TABLE I

THE EFFECTS OF VARIOUS UNLABELED AGENTS ON THE BINDING OF 20 μM [^{3}H]PHLORIZIN TO FAT CELL PLASMA MEMBRANES

Additions	*% of control bound*
None	100
Phlorizin, 1 mM	57
Glucose, 50 mM	100
Cytochalasin B, 0.1 mM	98
Phloretin, 0.3 mM	103
Phloretin, 1 mM	122

REFERENCES

1 LeFevre, P. (1961) *Pharmacol. Rev.* 13, 39
2 Alvarado, F. (1967) *Biochim. Biophys. Acta* 135, 483–495
3 Kuo, J.G., Dill, I.K. and Holmlund, C.E. (1967) *Biochim. Biophys. Acta* 144, 252–258
4 Crofford, O.B., Stauffacher, W., Jeanrenaud, B. and Renold, A.E. (1966) *Helv. Physiol. Acta* 24, 45–57
5 Czech, M.P., Lynn, D.G. and Lynn, W.S. (1973) *J. Biol. Chem.* 248, 3636–3641
6 LeFevre, P.G. and Marshall, J.K. (1959) *J. Biol. Chem.* 234, 3022–3026
7 Czech, M.P. and Lynn, W.S. (1973) *Biochim. Biophys. Acta* 297, 368–377
8 McKeel, D.W. and Jarett, L. (1970) *J. Cell Biol.* 44, 417–432
9 Czech, M.P. and Lynn, W.S. (1973) *J. Biol. Chem.* 248, 5081–5088
10 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) *J. Biol. Chem.* 193, 265–275
11 Malathi, P. and Crane, R.K. (1969) *Biochim. Biophys. Acta* 173, 245–256
12 Czech, M.P., Lynn, D.G., Mariencheck, W. and Lynn, W.S. (1973) *Clin. Res.* 21, 86 Abstr.