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THE INTERACTION OF 2-DEOXY-D-GLUCOSE AND GLUCOSE: EFFECTS ON THE SHORT-CIRCUIT CURRENT OF FROG SKIN

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Summary

The short-circuit current in frog skin is inhibited by 2-deoxy-D-glucose. Glucose interferes with this effect. When analyzed according to the Michaelis-Menten model the kinetics appear to be competitive.

The compound 2-deoxy-D-glucose has long been used as an inhibitor of glucose metabolism [1]. Among other effects it depletes the ATP level, and the phosphorylated form 2-deoxy-D-glucose 6-phosphate has been found to block the conversion of Glc-6-*P* to Fru-6-*P* [2]. We have observed an interaction between glucose and 2-deoxy-D-glucose with respect to the short-circuit current of the frog skin.

The ventral skin from one large frog (*Rana pipiens*, Carolina Biological Supply Co.) was divided in half longitudinally to provide experimental and control hemiskins, and each was mounted between the halves of a standard Ussing-Zerahn lucite chamber with 7.1 cm^2 cross-sectional area. Both the inside and outside surfaces of the hemiskins were bathed with 15 ml of aerated standard frog Ringer's solution [3]. Stock glucose and 2-deoxy-D-glucose solutions were made isosmotic with frog Ringer's solution by replacing NaCl with either glucose or 2-deoxy-D-glucose. Appropriate volumes of these stock solutions were added to the media bathing the skins to achieve the desired experimental concentrations. The voltage drop across the hemiskins was controlled by an automatic voltage clamp which permitted continuous recording of the short-circuit current I_0. After addition of a single dose of 2-deoxy-D-glucose to the inside solution, I_0 begins declining immediately reaching a lower steady-state value in some three-quarters of an hour. Fig. 1 shows the results of repeated additions of increasing amounts of 2-deoxy-D-glucose to a skin exposed to a concentration of 0.5 mM glucose.

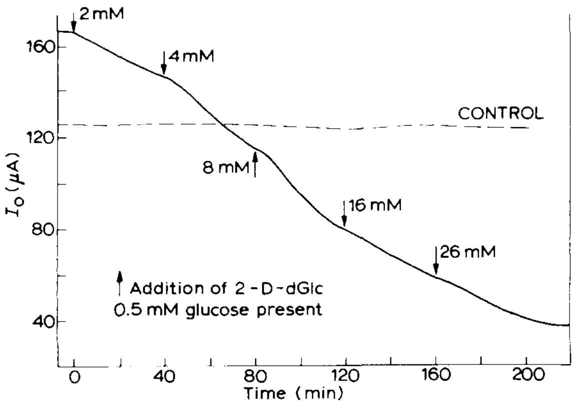

Fig. 1. The effect of various concentrations of 2-deoxy-D-glucose (2-D-dGlc) on short-circuit current (I_0) in frog skin. At the times indicated by the arrows the inside concentration of 2-deoxy-D-glucose was brought to the levels shown.

Several such experiments were performed using different concentrations of glucose. The results were analyzed by analogy with standard enzyme kinetics. The fractional current inhibition is given by

$$F = 1 - [I_0/I_{0_{t=0}}]_e/[I_0/I_{0_{t=0}}]_c \qquad (1)$$

where e stands for experimental and c for control hemiskins, and t denotes time of measurement. In analogy with the Michaelis-Menten equation we write

$$F = F_{max}\,[\text{2-D-dGlc}]/([\text{2-D-dGlc}] + K) \qquad (2)$$

The brackets denote concentration of 2-deoxy-D-glucose. F_{max} and K are the maximum fraction of inhibition and the concentration of 2-deoxy-D-glucose for which inhibition is half-maximal, both parameters being determined for a given concentration of glucose. Fig. 2 shows a Lineweaver-Burk plot of data such as those shown in Fig. 1.

TABLE I

INTERACTION OF 2-DEOXY-D-GLUCOSE AND GLUCOSE: ESTIMATES OF F_{max} AND K FOR INHIBITION OF SHORT-CIRCUIT CURRENT IN FROG SKIN, BASED ON A PLOT OF [2-D-dGlc]/F AGAINST [2-D-dGlc].

	0.1 mM	0.5 mM	1.0 mM	10 mM
F_{max}	0.759	0.995	0.785	1.71
S.E.	0.031	0.126	0.110	0.41
K(mM 2-D-dGlc)	5.4	11.4	21.6	28.7
S.E.	0.9	2.2	4.7	7.4
n	9	10	7	7

Linear transformations of Eqn 2 were fitted to the data by least squares. The most reliable estimates of F_{max} and K were determined for various glucose concentrations according to the criteria of Dowd and Riggs, plotting [2-D-dGlc]/F against [2-D-dGlc] (Table I) [4]. K appears to increase with increasing glucose concentration. The F_{max} data are not sufficiently precise to demonstrate any significant differences. Also, the standard least squares fitting of a linear transformation of a non-linear equation such as (2) can

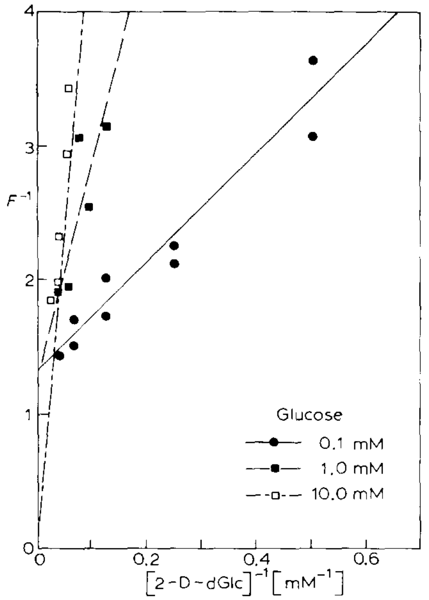

Fig. 2. Lineweaver-Burk plot of F, the fractional inhibition of short-circuit current in frog skin, by 2-D-dGlc in the presence of various concentrations of glucose. The lines were fitted by least squares. To conserve space a few high values of F^{-1} were omitted (2 with 1.0 mM glucose and 3 with 10.0 mM glucose).

lead to a non-normal distribution of the estimates of the parameters. The standard error estimates obtained from assuming a normal distribution do not then have an unambiguous interpretation [4,5]. However, it appears that Eqn 2 provides a useful working model of the 2-deoxy-D-glucose effect. The interaction between glucose and 2-deoxy-D-glucose seems to be competitive. F_{max} appears to lie between 0.75 and 1.

The availability of a method of reducing I_0 to any desired level by means of a metabolic inhibitor should prove useful in a number of areas. In particular, the technique has facilitated the analysis of a nonequilibrium thermodynamic formulation of active sodium transport [3].

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