Action of Insulin on Distribution of Glucose Analogs in Eviscerated-Nephrectomized Dogs

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ABSTRACT

LANDAU, BERNARD R., ARTHUR G. SHIP AND HERBERT J. LEVINE. Action of insulin on distribution of glucose analogs in eviscerated-nephrectomized dogs. Am. J. Physiol. 195(3): 461-465. 1958.—Insulin increased the distribution of 2-deoxyglucose, 3-deoxyglucose and 2-deoxygalactose in eviscerated-nephrectomized dogs. 6-O-methylglucose was nonresponsive to insulin. The response of 3-O-methylglucose appeared dependent upon the blood glucose level. These results are interpreted as indicating that the stereochemical requirements of aldohexoses for insulin response does not require a hydroxyl group on either carbon 2 or 3 of the molecule, nor does it require a specific configuration of the hydroxyl on carbon 4. It is suggested from the behavior of the methylated analogs that the size rather than the functional nature of the group at carbons 3 and 6 is important in determining insulin responsiveness.

In recent years increasing attention has been directed to the possible role of insulin in the transfer of glucose across the mammalian cell membrane. Levine and co-workers (1) concluded from studies of the distribution of a number of hexoses and pentoses, with and without insulin in the nephrectomized-eviscerated dog preparation, that the entrance of a sugar into muscle is governed by an insulin activated transfer system specifically adapted with respect to chemical structure. Responsive sugars were believed characterized by having the same configuration as glucose with respect to the first three carbon atoms. With few exceptions similar molecular configuration prerequisites for responsive sugars have been found by Drury and Wick (2) in eviscerate rabbits, and by Park and co-workers (3) in eviscerate rats. However, Helmreich and Cori (4), studying pentose distribution in the nephrectomized rat, could not correlate insulin responsiveness with a particular sugar structure.

Ross (5) has concluded that the evidence supports the existence of a specialized transport mechanism which facilitates the entry of glucose and certain other aldoses into the cells of extrahepatic tissues. While glucosamine and 2-deoxyglucose are exceptions to the stereochemical configuration requirements suggested by Levine, the configuration of the first, third and terminal carbon atoms are the same in all aldoses whose transport is known to be accelerated by insulin. Ross has suggested that there exists an enzymatically controlled transport system in which the function of the enzyme is to orient the glucose molecule, particularly carbon atoms 1 and 3 thereby facilitate binding to the carrier.

In the course of evaluating in eviscerated-nephrectomized dogs various deoxy and O-methyl aldohexoses as possible antimetabolites, we have obtained data which shed further light on the stereochemical requirements for insulin action. The analogs tested include 2- and 3-deoxyglucose, 3- and 6-O-methylglucose and 2-deoxygalactose.

MATERIALS AND METHODS

The evisceration-nephrectomy procedure was similar to that employed by Levine and co-workers (6). Twenty-two mongrel dogs of both sexes, weighing between 4 and 16 kg, and fasted 24 hours were used. Glucose was administered as a 2–3% solution in normal...
Fig. 1. Administration of insulin caused an increased distribution of 2-deoxyglucose, 3-deoxyglucose and 2-deoxygalactose as evidenced by decreased blood and plasma levels. No insulin effect was evident with 6-O-methylglucose. Blood glucose levels rose following infusion of 2-deoxyglucose.

e saline at 100-150 mg/kg/hr. by a Bowman constant infusion pump via a left femoral vein cannula. Blood samples were obtained from a cannulated right femoral artery. Blood pressure, pulse and respiratory rate were followed throughout the experimental period to assure the adequacy of the preparation. Hematocrits were obtained at frequent intervals.

The sugar was administered intravenously in doses of 200-1000 mg/kg as a 10-20% solution in normal saline over a 5-30-minute period. The length of time of administration depended upon the changes in vital signs. Doses of 500 mg/kg of 2-deoxyglucose even when administered slowly sometimes resulted in death of the dog. Glucagon-free insulin in doses of 5-15 u was administered intravenously between 1 1/4 and 2 hours after administration of the sugar derivative and beginning at that time insulin was added to the continuous infusion so that the dog received 1-3 u/kg/hr. Each sugar was tested in at least two dogs.

Levels of glucose, 3-deoxyglucose and 6-O-methylglucose were determined in whole blood and usually also in plasma by a modification of the glucose oxidase method of Wyngaarden, Segal and Foley (6a). The

2-deoxyglucose and 2-deoxygalactose whole blood and plasma levels were determined using Quinaldine Reagent (9). Using the Nelson blood sugar method (10) the 2-deoxy sugars on 20 minutes boiling had only one-twentieth the reducing value of glucose. Therefore glucose in the presence of these sugars was determined by measuring total Nelson reducing substance and making suitable small corrections for the reduction contributed by the quantity of 2-deoxy sugar present. By the Nelson method, 3-deoxyglucose and 3-O-methylglucose had approximately one-third and 6-O-methylglucose two-thirds the reducing power of glucose. Standard mixtures of the sugar analogs and glucose were tested to make certain the sugars would not interfere with one another in the determinations.

In several experiments, D-arabinose was infused at the same time as the sugar analog to provide a control known not to respond to insulin. Arabinose was determined by the method of Roe and Rice (11). Glucose present was determined by the glucose oxidase method and by subtracting the contribution of arabinose to the nonglucose reducing residual, the method of Keilin and Hartree (7) these findings were confirmed and 3-deoxyglucose and 2-deoxygalactose were found not to be oxidized. However as McComb et al. (8) recently reported, 2-deoxyglucose is oxidized by glucose oxidase at a considerable rate.

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1 Keilin had demonstrated that 3-O-methylglucose is not oxidized by glucose oxidase and 6-O-methylglucose only to a small extent. Using the incubation
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amount of glucose analog present was determined.

The 2-deoxyglucose was obtained from the Aldrich Chemical Company, Milwaukee, Wisconsin. It had been recrystallized from amyl alcohol, decolorized with charcoal, dried in vacuo, and been demonstrated to be chromatographically pure. The 3-O-methylglucose was prepared by the method of Glen (12). The 6-O-methylglucose was prepared by the method of Bell (13). The 2-deoxygalactose was prepared from galactal by the method of Overend (14). The 3-deoxyglucose was generously provided by Dr. James Pratt of the National Institutes of Health, Bethesda, Maryland.

RESULTS

In control studies galactose, glucose and D-arabinose were tested and found to respond as reported by Levine and Goldstein (1). The 2-deoxyglucose, 3-deoxyglucose, 3-O-methylglucose, 2-deoxygalactose, but not 6-O-methylglucose were responsive to insulin. This is shown graphically in figure 1. Sugar ‘space’ (6) before insulin administration was 25-35% of body weight; in the presence of insulin this space increased to 50-70% for 3-deoxyglucose and 3-O-methylglucose, and to 70-80% for 2-deoxyglucose and 2-deoxygalactose. Where plasma and whole blood levels were determined, the plasma level was found to be a more dramatic index of response. The approach of whole blood and plasma to the same levels of concentration also proved to be an indicator of response. Comparison of plasma level concentration with the calculated red blood cell concentration provided no evidence that insulin increased the rate of entrance of these sugar analogs into the red blood cells. 2-Deoxyglucose was administered after a control period during which the blood glucose level was constant. Following 2-deoxyglucose administration there was a significant rise in blood glucose although the infusion rate of administered glucose was unchanged.

The response of 3-deoxyglucose and 6-O-methylglucose in the presence of D-arabinose is shown in figure 2. D-Arabinose, as reported by others, did not respond to insulin. It occupied a sugar space of 25-35% of body weight. It thus served as a control for comparison of the response and distribution of the sugars tested just as glucose served as a control sugar known to be responsive to insulin.

The 3-O-methylglucose did respond at normal blood glucose levels to insulin (fig. 3a) but at high glucose levels it behaved as a nonresponsive sugar (fig. 3b). In figure 3c is graphed the results of an experiment in which following insulin administration in the presence of 3-O-methylglucose, an increased quantity of glucose was infused. There is a suggestion that there was initial response of the 3-O-methylglucose, but that further disap-

Fig. 2. D-arabinose occupied a sugar ‘space’ of 25-35% and did not respond to insulin. Note that in the case of a nonresponsive sugar the plasma level remained higher than the whole blood level. Glucose did respond to insulin administration. 3-Deoxyglucose response was more evident by the decrease in plasma than by the decrease in whole blood concentration. Following insulin administration it occupied a space of 50%. 6-O-Methylglucose which was nonresponsive occupied a space of 35%.
If pentoses do enter cells by the same mechanism as hexoses, then the configuration in the 5 position cannot be significant since in the pentoses the 5th carbon is not asymmetric. Similarly in the hexoses there can be no configurational requirement for carbon 6. The effect of the absence of the hydroxyl on carbon 5 in the pentoses and carbon 6 in the hexoses will have to await studies with these deoxysugars.

Drury and Wick (2) in the rabbit found 3-O-methylglucose to be unresponsive (personal communication from A. N. Wick). We have evidence that it is responsive, but that the response is dependent on the blood glucose level. This observation is in agreement with the observed competition between glucose and 3-O-methylglucose for penetration into Ehrlich ascites tumor cells (17).

Glucose and galactose are known to be responsive sugars. That their 2-deoxy derivatives also respond lend support to the conclusion that the configuration of the hydroxyl at carbon 4 does not help to determine insulin responsiveness.

Various structural requirements of aldoses for insulin responsiveness in the eviscerated-nephrectomized dog can be formulated:

The aldehyde group representing carbon 1 of glucose, not having an asymmetric carbon atom, can offer no stereochemical obstacle to response. However, as the hemiacetal, as suggested by Keston (15), the alpha or beta configuration might be preferred. In aqueous solution, most of the sugars tested are known to rapidly obtain alpha-beta equilibration.

Wick (16) demonstrated that in the eviscerated-nephrectomized rabbit insulin appeared to accelerate the rate of entry of 2-deoxyglucose into cells although positive conclusions as to the insulin effect were felt to require more sensitive methods of determination. It is now apparent that since both 2-deoxyglucose and 2-deoxygalactose are responsive sugars, the hydroxyl in the two position is not required.

The hydroxyl in the three position is not required as evidenced by the response of 3-deoxyglucose. Drury and Wick (2) in the rabbit found 3-O-methylglucose to be unresponsive (personal communication from A. N. Wick). We have evidence that it is responsive, but that the response is dependent on the blood glucose level. This observation is in agreement with the observed competition between glucose and 3-O-methylglucose for penetration into Ehrlich ascites tumor cells (17).

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Thus aldohexoses respond to insulin in the absence of a functional group in either the 2 or 3 position and the configuration of the hydroxyl in the 4 position does not determine insulin response. The apparent competition between glucose and 3-O-methylglucose for entrance into the extrahepatic tissues, and the failure of 6-O-methylglucose to respond despite the fact that it possesses the glucose configuration in its entirety, suggests that steric hindrance participates significantly in the determination of responsiveness. That is, the size, rather than the nature of the functional group, at least in the instances of position 3 and 6, is a determining factor. If indeed, a substrate-carrier system, as suggested by Ross (5) does exist, one may picture the aldohexose ‘fitting’ on the carrier in such a manner that increasing the size of the group in position 3 or position 6 interferes with the ‘fit’ and inhibits or prevents the carrier system from functioning. However removal of the hydroxyl at position 2 or 3 still permits the
sugar to fit in the space allotted on the carrier and therefore the system is still effective.

The observed rise in glucose level following 2-deoxyglucose administration is in agreement with Wick’s observation (16) of decreased glucose oxidation in the rabbit following 2-deoxyglucose administration.

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REFERENCES